CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 34 MAY-JUNE 1954 No. 3

STUDIES ON HUMUS TYPE LEGUME INOCULANTS

II. PREPARATION AND EFFECTIVITY[1]

C. GUNNING[2] AND D. C. JORDAN[3]

Ontario Agricultural College, Guelph, Ont.

[Received for publication July 29, 1953]

ABSTRACT

Studies were carried out on humus type legume inoculants with particular reference to production, and the longevity and effectiveness of the organisms present. Lochhead's nutrient solution in the original form and in various modifications, constantly aerated, produced higher cell counts than the other media tested.

Under the experimental conditions used all the cultures, after one year of storage, contained sufficient nodule bacteria for seed inoculation purposes. Storage at 5° C. was preferred when long storage periods were to be employed, but perfectly satisfactory results were obtained with cultures maintained at room temperature. All the cultures were shown to be effective in nitrogen fixation after 6 months' storage, under controlled conditions and after 3 months' storage under field conditions. Refrigerated cultures tested in the greenhouse were superior to room temperature cultures as measured by the dry weights of their host plants. This difference, however, was not considered sufficient to advocate the refrigeration of all cultures.

INTRODUCTION

Newbould (7) in 1951 reported a method for the preparation of a humus type legume inoculant which would maintain large numbers of viable rhizobia throughout a storage period of four months. Such cultures consisted of a mixture of a specific bacterium, a nutrient solution, and a peat carrier, and were contained in sealed metal cans. The foregoing work, however, can only be regarded as preliminary, since before the release of any new culture for distribution critical testing procedures must be carried out, especially relative to the efficiency of such inoculants in nitrogen fixation.

The present investigation deals with humus cultures particularly from the standpoint of medium selection, mode of preparation and the longevity and efficiency of the contained bacteria.

[1] Contribution from the Department of Bacteriology, Ontario Agricultural College, Guelph, Ont. Part of the program of the Legume Research Committee in Ontario.

[2] Assistant in Research.

[3] Lecturer in Bacteriology.

EXPERIMENTAL

Medium Selection

Methods

For the preparation of humus type inoculants in large quantities it was necessary to determine the best media and methods by which heavy concentrations of rhizobia can be propagated. Of the various media which have been used for this purpose, those of Albrecht and McCalla (1) and of Lochhead* appeared most suitable. These media, in their original forms and with various deletions and supplements, constituted the substrates investigated. The formula of Lochhead's medium (soybean) is as follows: (*a*) agar, 15 gm.; tap water, 600 ml.; (*b*) potassium dihydrogen phosphate, 0.4 gm.; magnesium sulphate, 0.1 gm.; sodium chloride, 0.1 gm.; calcium sulphate, 0.05 gm.; calcium carbonate, 2.0 gm.; tap water, 200 ml.; (*c*) sucrose, 10 gm.; mannitol, 4 gm.; maltose, 2 gm.; yeast extract, 1 gm.; malt extract, 0.25 gm.; tap water, 200 ml. The three solutions are prepared separately, dissolved in a steamer, and then mixed together. In the Lochhead's modified medium the mannitol, maltose, and malt extract are omitted.

The actual propagation of the organisms was carried out in either an aerated culture system (7), or in a charcoal-cellophane dialyzing system as described by Gorelick *et al.* (2). The latter method was abandoned after a brief trial, primarily because only a small volume of inoculum could be produced.

The test organisms, representative of four major cross-inoculation groups, are listed in Table 1, together with the media and the propagation techniques used.

TABLE 1.—TABLE SHOWING TEST ORGANISMS, MEDIA, AND METHODS USED

Organism	Medium	Method
Rh. meliloti R_{21}	(*a*) Lochhead's modified	Aeration
	(*b*) Lochhead's modified plus an amino acid complex	Aeration
	(*c*) Albrecht and McCalla's	Aeration
	(*d*) Albrecht and McCalla's	Charcoal-cellophane
Rh. japonicum R_{4A}	(*a*) Lochhead's soybean	Aeration
	(*b*) Lochhead's soybean (arabinose for sucrose)	Aeration
	(*c*) Lochhead's soybean (arabinose for sucrose; maltose and mannitol omitted)	Aeration
	(*d*) Albrecht and McCalla's	Aeration
	(*e*) Albrecht and McCalla's plus yeast extract (arabinose for sucrose)	Aeration
Rh. trifolii R_{26}	(*a*) Lochhead's modified	Aeration
	(*b*) Albrecht and McCalla's	Aeration
Rh. leguminosarum R_{3}	(*a*) Lochhead's modified	Aeration
	(*b*) Albrecht and McCalla's	Aeration

*Lochhead, A. G. *Personal communication.*

The amino acid complex added to Lochhead's modified medium consisted of the following, each at a final concentration of 10 mgm./litre: dl-methionine, glycine, l-cystine, l-histidine monohydrochloride, l-arginine, l-glutamic acid, l-aspartic acid, l-proline, and asparagine. Leucine (dl) was added in a final concentration of 20 mgm./litre. This complex was added, in addition to yeast extract, in an effort to eliminate any undue lag and thus promote more rapid growth (5).

Arabinose was substituted for sucrose in several cases where Lochhead's medium was used because it is claimed to be distinctly superior to other carbonaceous compounds as an energy and carbon source for soybean rhizobia (6). In one case mannitol and maltose were excluded, on the grounds that they are used only sparingly by this organism (6).

At 24-hour intervals, following inoculation of the flasks, the bacterial populations were determined by a standard plating technique. Either Lochhead's modified medium ($CaCO_3$ omitted) or Lochhead's soybean medium ($CaCO_3$ omitted), depending upon the *Rhizobium* species to be enumerated, was used for plating purposes, regardless of whether quantitative studies were being made on inocula produced in Lochhead's or in Albrecht and McCalla's solution. Statistical analysis showed that this single factor had no significant detrimental effect upon the accuracy of the counts made on cells grown in the latter nutrient solution.

Results and Discussion

The propagation studies with the alfalfa *Rhizobium* R_{21} in the various nutrient solutions (Figure 1) indicated a marked superiority of Lochhead's modified medium over Albrecht and McCalla's medium, particularly

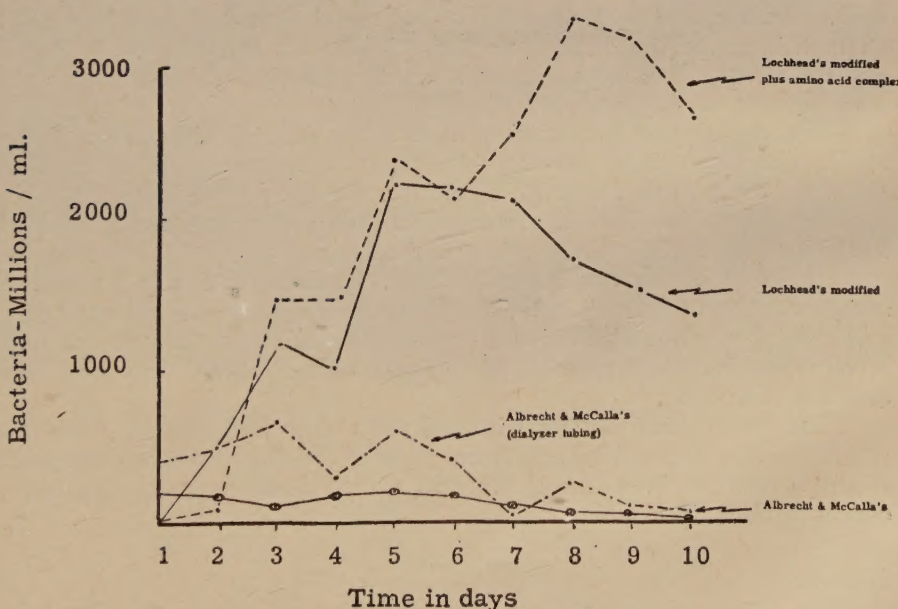


FIGURE 1. Propagation of an alfalfa root-nodule organism in various nutrient solutions in aeration flasks and by a charcoal-cellophane system.

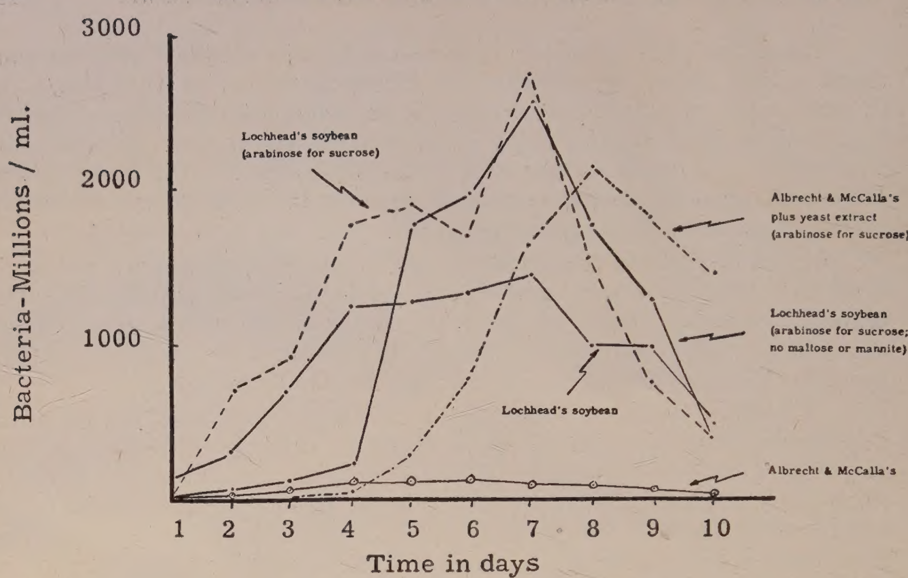


FIGURE 2. Propagation of a soybean root-nodule organism in various nutrient solutions in aeration flasks.

when the amino acid supplement was added. The growth curve of the cells in the charcoal-cellophane arrangement is misleading, in that it suggests multiplication of the cells to a concentration appreciably in excess of that in the aeration flasks containing the same substrate. Actually, the count after one day is higher in the former only because the initial inoculum was diluted to a lesser degree in this container than in the aeration flask. Lochhead's soybean medium surpassed Albrecht and McCalla's medium in supporting heavy growth of *Rhizobium* R_{4A} (Figure 2), but it was the altered forms of Lochhead's soybean solution which produced the most concentrated growth.

The omission of mannitol and maltose from Lochhead's soybean medium in which arabinose was substituted for sucrose appeared to affect the resulting growth curve by extending the lag period throughout the first four days of incubation. No significant difference was found, however, in the maximum cell concentration reached.

Although Albrecht and McCalla used sauerkraut juice as a source of accessory growth factors in their medium, the inclusion of yeast extract and the substitution of arabinose for sucrose resulted in the production of a cell mass of soybean rhizobia far in excess of that obtained in the parent medium (Figure 2).

The propagation studies with the red clover *Rhizobium* R_{26} and the pea *Rhizobium* R_3 provided additional proof of the superiority of Lochhead's modified medium over that of Albrecht and McCalla for producing heavy concentrations of rhizobia.

Longevity

Methods

The equipment used to dispense and mix the culture ingredients is shown in Figure 3. Upon depressing the hand lever a measured volume of

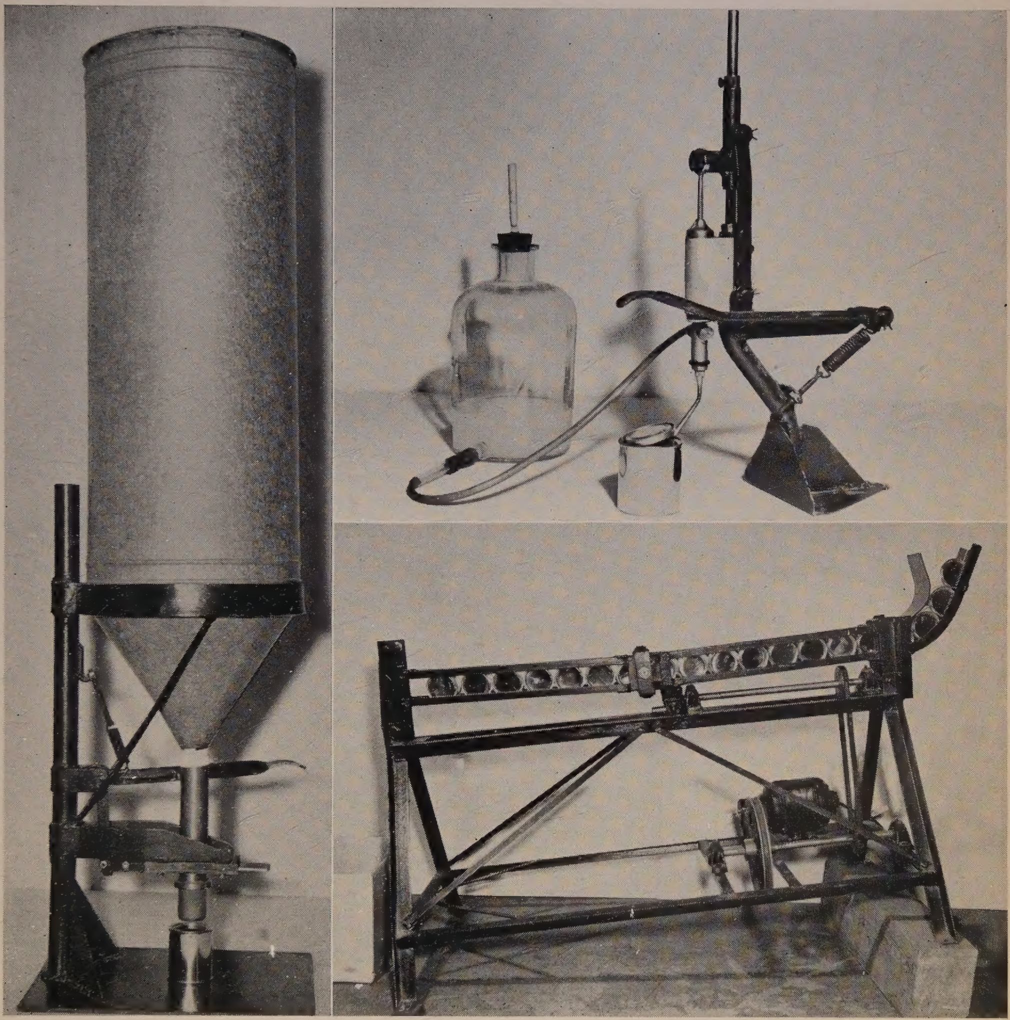


FIGURE 3. Equipment for dispensing and mixing the culture ingredients.
Left: Peat dispenser.
Top right: Inoculum dispenser.
Bottom right: Power-driven rotating mixer.

air-dried peat or peat containing 10 per cent wood charcoal (7), was dispensed from the metal peat reservoir into one-half pint paint containers. Loosely capped, the cans and their contents were sterilized in a hot air oven at 170° C. for one hour. Inoculum and fresh nutrient solution were then mixed in a ratio of 1 : 4 and a calculated volume of this suspension was added to the sterilized peat. The fluid dispenser used consisted of a special two-way valve fitted to a veterinary syringe and was designed to draw a measured volume of fluid from a reservoir on the upstroke of the lever and to deliver this aliquot on the down stroke. The diluent performed the two-fold duty of raising the moisture level of the dried peat to 50 per cent of its water holding capacity, an amount considered optimum for the survival of the bacteria (7) and for providing an adequate food reserve for the culture throughout storage. The dispenser was easily 'sterilized' by flushing with a solution containing 200 p.p.m. available chlorine, followed by rinsing with sterile water. The inoculum and carrier were then thoroughly mixed by rolling the closed cans through the rapidly rotating, centrally jointed shaft of a power mixer.

The culture groups tested, together with other pertinent information, are given in Table 2. Duplicate lots were prepared and stored at room temperature and at 5° C. The cultures stored at 5° C. were held at room temperature for 24 hours after preparation and prior to refrigeration. The cultures containing Fred and Waksman's and Albrecht and McCalla's nutrient solutions, and those containing ordinary peat as the carrier, were of the type prepared by Newbould (7). It was felt advisable to further test these cultures since preliminary work had shown them to be capable of maintaining large numbers of viable rhizobia for periods up to four months.

Counts were made at monthly intervals by means of standard plating methods, a fresh can being used each time. The triplicate plates were

TABLE 2.—TABLE LISTING DATA PERTINENT TO THE TESTING OF POWDER INOCULANTS

Series	Group	Organism	Nutrient solution	Carrier	Storage period
1	A	*Rh. meliloti* R_{21}	Fred and Waksman's	Peat	
1	B	*Rh. meliloti* R_{21}	Albrecht and McCalla's	Peat	2 and 6 months
1	C	*Rh. meliloti* R_{21}	Fred and Waksman's	Peat + 10% charcoal	
1	D	*Rh. meliloti* R_{21}	Albrecht and McCalla's	Peat + 10% charcoal	
2	A	*Rh. japonicum* R_{505}	Lochhead's soybean	Peat + 10% charcoal	6 months
5	A	*Rh. meliloti* R_{21}	Albrecht and McCalla's	Calcareous peat + 10% charcoal	6 months
5	B	*Rh. meliloti* R_{21}	Lochhead's modified	Calcareous peat + 10% charcoal	

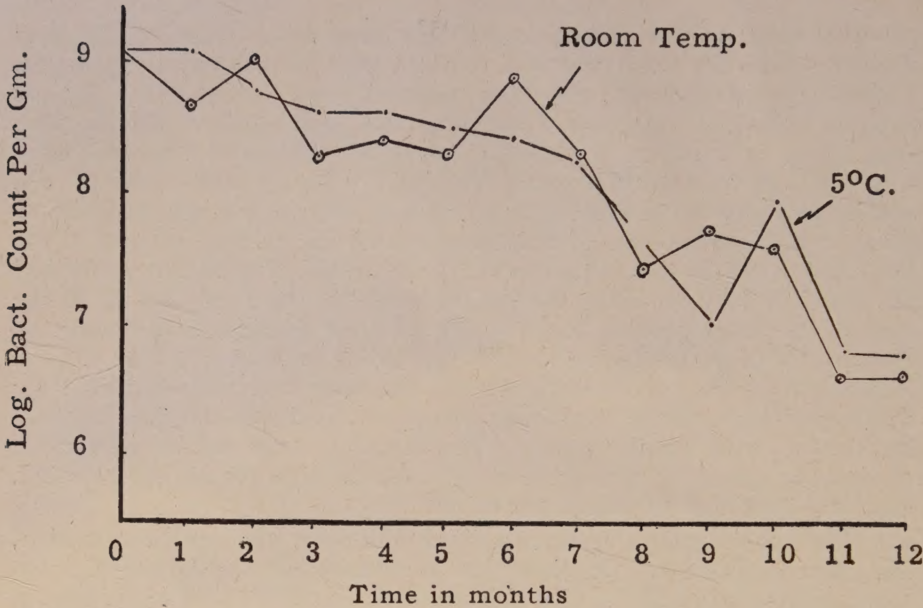


FIGURE 4. A comparison of the survival of the soybean Rhizobium R_{505} at different temperatures in closed cans of powdered peat supplemented with 10 per cent wood charcoal and Lochhead's soybean nutrient solution.

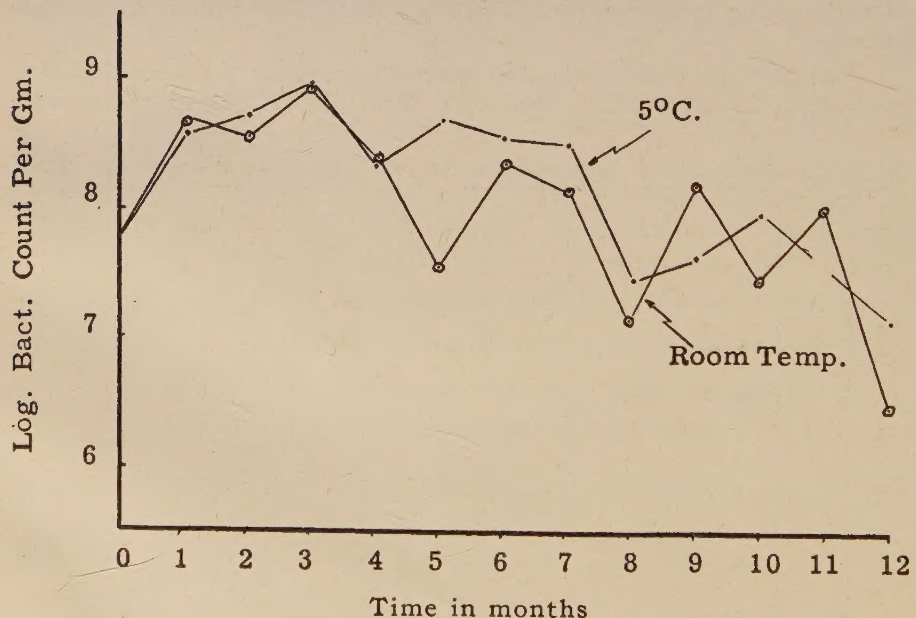


FIGURE 5. A comparison of the survival of the alfalfa Rhizobium R_{21} at different temperatures in closed cans of powdered calcareous peat supplemented with 10 per cent wood charcoal and the nutrient solution of Albrecht and McCalla.

counted after 6 days' incubation at 25° C. and the accuracy of all plate counts was controlled by the use of the index of dispersion (D^2) and the control chart of Hannay (3).

Results and Discussion

The cultures as used by Newbould (7) showed satisfactory survival of the rhizobia over a 12-month period. Figures 4, 5, and 6 show the graphs of the bacterial counts in the new cultures after a similar length of time.

It was apparent that the majority of cultures followed the same general growth and survival pattern, the peak populations being reached by the fourth month. The most important feature is the fact that the cell counts of the cultures stored at 5° C. closely paralleled or surpassed those of their counterparts stored at room temperature. For an organism having an optimum growth temperature of 25°-30° C. such a finding warranted further investigation.

After determining their initial cell counts, cultures of the soybean *Rhizobium* R_{505} were stored continually at room temperature, at 5° C. following 24 hours at room temperature, or continually at 5° C. According to periodic viable cell counts the increase of organisms to the vicinity of 10^9 cells per gram of carrier appeared to be reached within the first 48 hours (Figure 7). Refrigerating the second culture after 24 hours at room temperature did not immediately arrest the multiplication of the organisms, a fact which may possibly be explained by an insulating effect of the peat. By the first week, however, there was a decided drop in the bacterial count of this culture, only to be followed by a steady cell multiplication, until by the second month the count surpassed that of the then declining culture

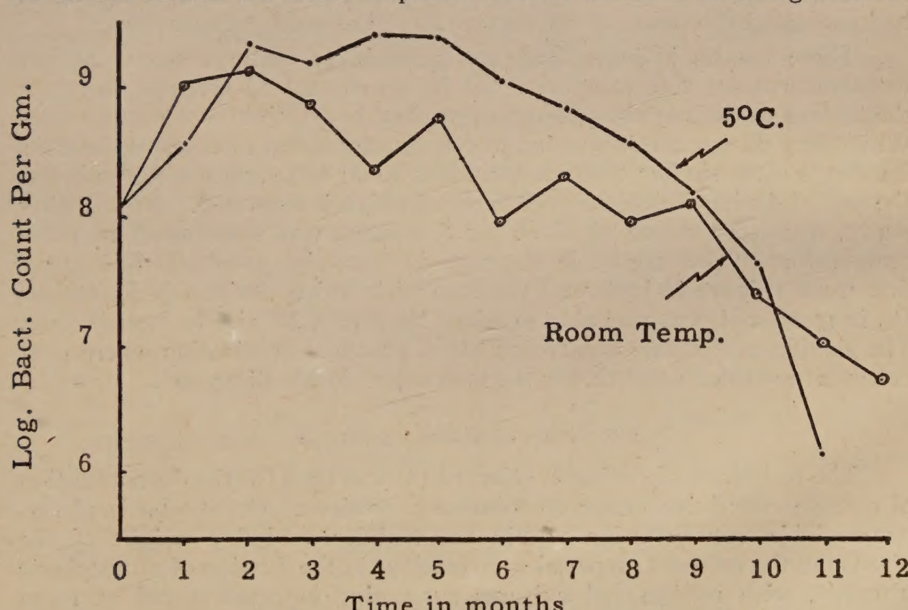


FIGURE 6. A comparison of the survival of the alfalfa Rhizobium R_{81} at different temperatures in closed cans of powdered calcareous peat supplemented with 10 per cent wood charcoal and Lochhead's modified nutrient solution.

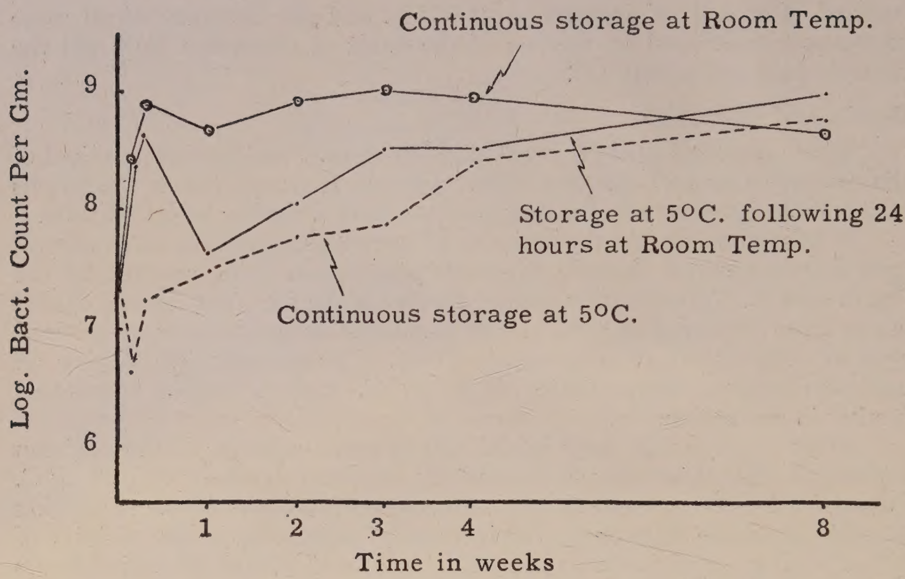


FIGURE 7. A comparison of the initial portions of the survival curves of the soybean Rhizobium R_{505} at different temperatures in closed cans of powdered calcareous peat supplemented with Lochhead's soybean nutrient solution.

stored at room temperature. The bacterial count of the 5° C. culture immediately dropped, but in 48 hours the organisms had recovered from the initial shock and were beginning to increase, and, by the second month, had surpassed the count of the culture stored at room temperature.

These results indicate that the rhizobia in cultures stored at low temperatures are able to survive the initial shock and are able to adapt themselves to their environment and proceed to multiply at a regular rate. While they do not attain maximum cell concentration until approximately 2 months after similar cultures stored at room temperature, nevertheless the period during which the counts remain high is extended. In a further study, a culture stored at 5° C. for 5 months was transferred to room temperature. The negligible decrease in bacterial numbers during the first week (Figure 8) indicated the ease with which the alfalfa *Rhizobium* R_{21} in powdered peat was able to adapt itself to a 20° rise in temperature. The significance of this occurrence has a practical application where peat cultures are stored at 5° C., for the reason previously discussed.

Effectivity of Humus Cultures

The technique of Jordan & Garrard (4) was used for the determination of culture effectivity under greenhouse conditions. On the basis of leg-haemoglobin concentration within the nodules, and dry weight of the plants, all the cultures prepared were efficient in the fixation of atmospheric nitrogen, with refrigerated cultures surpassing cultures stored at room temperature in this respect.

Field trials were carried out at three areas locally differing in drainage, soil type and previous crops. The locations were laid out in Latin squares

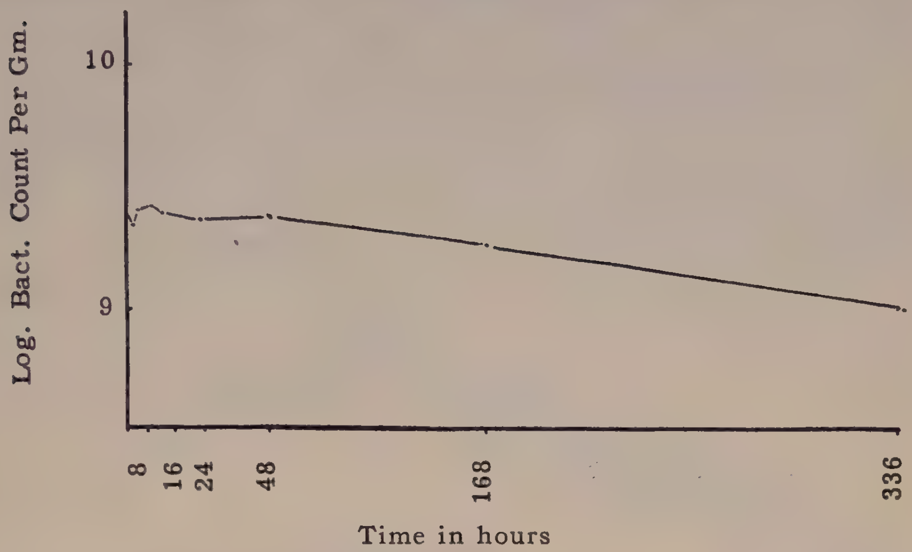


FIGURE 8. The survival of the alfalfa Rhizobium R_{21} in a closed can of powdered calcareous peat supplemented with 10 per cent wood charcoal and Lochhead's modified nutrient solution upon transfer to room temperature after 5 months at 5° C.

consisting of three replicate plots each of uninoculated Grimm alfalfa seed, seed treated with stored culture (3 months old), and seed treated with stored culture plus skimmilk. Both the peat inoculum and the peat inoculum plus skimmilk brought about a significant increase in the dry weights of the inoculated plants over the uninoculated controls, but no difference was apparent between the plant yields inoculated by the two different methods.

ACKNOWLEDGMENTS

The authors wish to acknowledge the kind assistance of J. R. Scott and J. Turnbull, of the Agricultural Engineering Department, Ontario Agricultural College, who designed and manufactured the equipment used in this investigation. The kind donation of powdered peat from the Humar Corporation of Ontario is also greatly appreciated.

REFERENCES

1. Albrecht, W. A., and T. M. McCalla. A new culture medium for rhizobia. J. Bact. 34 : 455-457. 1937.
2. Gorelick, A. N., D. D. Mead, and E. H. Kelly. The growth of bacteria in a charcoal-cellophane system. J. Bact. 61 : 507-513. 1951.
3. Hannay, C. L. A control chart for bacterial colony counts. Proc. Soc. Applied Bact. 2 : 85-88. 1946.
4. Jordan, D. C., and E. H. Garrard. Studies on the legume root nodule bacteria. I. Detection of effective and ineffective strains. Can. J. Botany 29 : 360-372. 1951.
5. Jordan, D. C. Studies on the legume root nodule bacteria. III. Growth factor requirements for effective, ineffective and parasitic strains. Can. J. Botany 30 : 693-700. 1952.
6. Neal, O. R., and R. H. Walker. Physiological studies on *Rhizobium*. IV. Utilization of carbonaceous material. J. Bact. 30 : 173-187. 1935.
7. Newbould, F. H. S. Studies on humus type legume inoculants. I. Growth and survival in storage. Sci. Agr. 31 : 463-469. 1951.

THE CAROTENOID PIGMENTS OF THE UREDOSPORES OF RUST FUNGI[1]

G. N. IRVINE[2], M. GOLUBCHUK[3] AND J. A. ANDERSON[4]

[Received for publication July 28, 1953]

ABSTRACT

The carotenoid pigments have been extracted from the following types of commonly occurring rust spores: wheat stem rust, wheat leaf rust, barley stem rust, oat stem rust, oat crown rust and flax rust. Chromatographic analyses suggested that the pigment composition of all six types is similar. The major pigment is probably γ-carotene with lesser amounts of lycopene and β-carotene being present. Identity of the latter two pigments was confirmed by mixed chromatogram. Some differences were found in the quantitative distribution of these pigments among the types of rust spores.

Though wide differences occur in the colour of the uredospores of various rusts indigenous to the cereal crops of Western Canada, information on the identity and distribution of the pigments in rust spores is meagre. The principal study reported in the literature is that of Newton and Johnson (6) done in 1927. They examined wheat stem rust and concluded that the pigment extracted by acetone or carbon disulphide was probably carotene. In a further short note, Newton, Johannson and Johnson (7) examined four colour variants of wheat stem rust and again concluded that the pigment was chiefly carotene.

Lederer (4), in a study of the carotenoid pigments of various fungi in 1934, reported that the uredospores of *Puccinia coronifera* (oat crown rust) contained γ-carotene and a mixture of α- and β-carotene. This is again confirmed in a later paper (5).

The present study is of an exploratory nature and was undertaken to ascertain what differences, if any, existed among the pigments of the different species of rust spores encountered in Western Canada. The various rust types show quite striking variations in spore colour. At the same time an attempt was made to re-examine and extend the information on the carotenoid pigments of rust spores. Since the earlier work of Newton *et al.* (6, 7) advances in methods and equipment have been substantial, and it appeared likely that more useful basic information could be obtained which would aid in the physiological characterization of the cereal rusts. The amounts of fresh spore material available for study were very small, most of it being grown on hosts in the greenhouse. Since it appeared from preliminary work that no substantial amounts of the pigments could be obtained for rigorous purification and study, elaborate preparation of solvents and other time-consuming refinements were not considered worth while.

---

[1] Joint contribution from the University of Manitoba and the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg. Paper No. 132 of the Grain Research Laboratory and No. 310 of the Associate Committee on Grain Research (Canada); with financial assistance from the National Research Council.

[2] Chemist.

[3] Graduate Student.

[4] Chief Chemist, Board of Grain Commissioners.

MATERIALS AND METHODS

During the course of this investigation six samples of wheat stem rust, three samples of wheat leaf rust, five samples of oat stem rust, three samples of oat crown rust, two samples of stem rust from barley and one sample of flax rust were examined. Most were of mixed races, but two stem rust samples represented the pure races 15 B and 121. The latter is a greyish brown mutant of normal stem rust.

Up to 20 grams of spores were available in some cases but this had been stored for several years and produced poor yields of pigment. In most instances fresh spore material available varied from 700 mgm. to 2.5 grams.

The solvents and adsorbents used in this study were all reagent grade chemicals and were not further purified. Absorption measurements were made with a Beckman Model DU spectrophotometer.

Extraction of the Pigments

It was not possible to obtain any pigment from unground spores of any age, either newly harvested or stored for several years, by extraction with any of the usual organic solvents. Grinding in a mortar with sand in the presence of ethyl ether yielded a deep yellow pigment solution with fresh spore material but not with spores that had been stored for any considerable length of time. Spores were digested with aqueous sodium hydroxide, alcoholic potassium hydroxide, and dilute acetic acid without success. A method which finally yielded satisfactory results in releasing the spore pigments from older material was a one-hour digestion with normal hydrochloric acid at 100° C. followed by centrifugation and extraction of the residual spore material with ethyl ether. This technique also resulted in a further release of pigment from the fresh material of a somewhat greater amount that was obtained from the initial extraction with sand grinding. Accordingly, the generalized technique for extraction was first to grind with sand and ether, and then to hydrolyze with normal acid and extract again with ether.

The use of the acid hydrolysis in this method raises the probability that the original pigments were altered by this treatment. Fortunately it was possible to obtain a fair amount of the pigment from fresh material without resorting to this treatment, and comparison of the results with and without acid hydrolysis indicated that identical chromatograms were obtained; from these, the same pigment fractions having the same absorption maxima were isolated. As a result, the two extracts, where both could be obtained, were usually combined before chromatographing.

Chromatographic Analysis

The rust spore pigments were found to be entirely epiphasic. Treatment of the material with acid as outlined above was considered to be sufficient to hydrolyze any xanthophyll esters which might be present; upon partitioning the crude pigment extract between petroleum ether and 90 per cent methanol all the coloured material migrated to the petroleum ether layer, indicating that the pigments were hydrocarbons rather than hydroxy carotenoids. Although various combinations of adsorbents, solvents and eluants were used during the investigation, the most satis-

factory was found to be magnesium oxide as adsorbent, petroleum ether (Skellysolve F) as solvent, and 20 per cent benzene in petroleum ether as eluting agent. The pigments were removed from the chromatogram by extrusion of the column and mechanical separation of the bands.

RESULTS

The same carotenoid pigments appeared to be present in all species of rust spores examined. Gamma carotene was the principal pigment found, with lesser amounts of β-carotene and lycopene. Some differences were found in the quantitative distribution of these pigments among the various species of rust. Material which had been stored for more than several months yielded considerably less pigment than freshly harvested spores.

The general type of chromatogram obtained from extracts of all species contained three coloured zones: a thin pink band, a wide orange band and a narrower yellow band, in order from the top of the column. The thin pink band contained the lycopene which showed a strong tendency to oxidize upon extrusion from the column and subsequent elution; it gave the greatest difficulty when attempts were made to rechromatograph. The other two bands were more readily removed, and rechromatographing in most instances indicated that the zones were homogeneous and contained a single pigment. The highest values obtained during the investigation for the absorption maxima of the pigments from each of the three zones are given in Table 1.

Table 1.—Absorption maxima for the rust spore pigments

Zone	Absorption maxima, mμ	
Zone 1	In carbon disulphide	542, 507
Zone 2	In carbon disulphide	532, 498.5 (467)
	In chloroform	507, 476
	In benzene	505, 475
	In petroleum ether (Skellysolve F)	489.5, 460, 433
	In ethanol	490.5, 459.5
Zone 3	In carbon disulphide	510, 484.5
	In benzene	490, 462
	In petroleum ether (Skellysolve F)	473, 447

These results were obtained upon rechromatographing the three pigment zones separated from the largest sample of freshly harvested spores (5 grams) used in the investigation. The ranges of the absorption maxima obtained in carbon disulphide for each of these zones in other experiments, all involving considerably less material, and in many cases older spore material, were as follows:

Zone 1—542, 507 mμ to 535, 505 mμ
Zone 2—532, 498.5 mμ to 525, 492 mμ
Zone 3—510, 485 mμ to 508, 481 mμ

Accordingly it would appear that in most cases with the smaller extracts, considerable isomerization took place, particularly for the Zone 1 and Zone 2 pigments; the principal maximum obtained in most cases for Zone 2 was 529 mμ (in CS_2) which corresponds with that for neo-γ-carotene U. Further evidence of this is apparent in Table 1 where the maxima for the Zone 2 pigment are 1.5 mμ lower than those for crystalline γ-carotene in both carbon disulphide and chloroform, but the values in the solvents used later in the experiment (on the same pigment sample) are about 5 mμ low. These lower values correspond with those for neo-γ-carotene U, indicating that isomerization occurred during the process of evaporating solvents and redissolving in others.

From its behaviour on partitioning, its colour and position on the adsorption column, and its absorption spectrum, the only reasonable identity for the Zone 1 pigment seemed to be lycopene. To confirm this lycopene was extracted from ripe tomatoes and purified by chromatography. The absorption maxima for this lycopene sample in carbon disulphide were 543, 506.5 (480) mμ, somewhat lower than the currently reported values for the crystalline pigment (548, 507.5, 477 mμ). A mixed chromatogram using this material and the Zone 1 rust spore pigment indicated that the two were identical.

The pigment from Zone 3 behaved in a manner characteristic of β-carotene. Commercial carotene (90 per cent β-, 10 per cent α- carotene was chromatographed and the β-carotene fraction removed. It exhibited absorption maxima at 508.5, 483 mμ. A mixed chromatogram indicated that the Zone 3 pigment was identical with the β-carotene separated from the commercial mixture.

The major pigment, from Zone 2, presented a more difficult problem in identification. Its absorption maxima corresponded closely with those reported for rubixanthin, gazaniazanthin, myxoxanthol and aphanin. Otherwise, from its colour, its position on the chromatogram, and its behaviour on partitioning with mixtures of aqueous methanol and petroleum ether, it appeared to be γ-carotene. A mixture was prepared of this rust spore pigment, lycopene, α-carotene and β-carotene. Upon chromatographing, the rust spore pigment was adsorbed between lycopene and β-carotene as had been found for the chromatograms of the crude rust spore pigments. According to Karrer and Jucker (3) only γ-carotene is adsorbed between lycopene and β-carotene. It was found possible to eliminate the other four possibilities on the basis of their behaviour on partitioning between 95 per cent methanol and petroleum ether. Since it is well known that natural pigments isolated from chromatograms rarely exhibit the same absorption maxima as solutions of the crystalline pigment, it seems reasonable to disregard the small differences in maximum between the major rust spore pigment (532, 498.5, 467 mμ) and crystalline γ-carotene (533.5, 496, 463 mμ), as all other evidence leaves little doubt that the two pigments are identical.

Some quantitative estimates of the relative amounts of the pigments in the various species of rust spores were made using Goodwin's values (2) for the molecular extinction coefficients of lycopene, γ-carotene and β-carotene; these estimates are given in Table 2. In all but one experiment

TABLE 2.—QUANTITATIVE ESTIMATES OF INDIVIDUAL PIGMENTS IN VARIOUS SPECIES OF RUST SPORES

Type of rust	Lycopene, p.p.m.	γ-carotene, p.p.m.	β-carotene, p.p.m.
Wheat stem rust	—	490	210
Wheat leaf rust	220	1130	360
Oat crown rust	—	1090	370
Flax rust	—	780	120

oxidation made it impossible to recover the lycopene fraction in sufficient quantity to give a reliable estimate; accordingly, lack of a figure for lycopene in the table does not indicate that it was not present.

The considerable differences in colour which exist among fresh spores of the various species are apparently not a result of wide differences in the amounts of the several pigments present. It appears rather that the exterior spore wall, which in general appears to have a brownish colour, is chiefly responsible for determining the bulk colour of the spores. Flax rust, which is a bright orange red, apparently has more transparent spore walls than the very deep brick red coloured leaf or stem rusts. Examination of the various species under the microscope does not indicate significant differences in spore colour; it is the mass effect of small differences between spore types that seems to result in the wide visible differences in colour.

It is not yet known what physiological significance is to be attributed to the extremely high concentration of carotenoid pigments present in fresh rust spore material. Presence of the three major components, distributed in the manner indicated in Table 2, seems reasonable on the basis of one current theory of carotenogenesis (1). Lycopene and β-carotene are symmetrical molecules, having the same grouping on either end of the central polyene chain. Gamma carotene is assymetric, having a lycopene residue on one end of the chain and a β-carotene residue on the other end. Accordingly it seems probable that any synthetic process which resulted in the production of large amounts of γ-carotene would result also in the synthesis of smaller amounts of lycopene and β-carotene.

The presence of these pigments is not essential for the development of the spores. One of the samples studied was a freshly harvested greyish brown colour mutant of wheat stem rust, race 121, which was found to contain no significant amount of any of the carotenoid pigments. It may be significant, however, that this species is extremely difficult to grow on wheat.

ACKNOWLEDGMENT

The authors wish to express their appreciation to T. Johnson of the Dominion Laboratory of Plant Pathology, Winnipeg, and G. A. Ledingham, of the Prairie Regional Laboratory, Saskatoon, for furnishing the rust spore samples. One of the authors (M.G.) is also grateful to the National Research Council for financial assistance.

REFERENCES

1. Bonner, J. Plant biochemistry. Academic Press, New York. 1950.
2. Goodwin, T. W. The carotenoids of the berries of *Lonicera japonica*. Biochem. J. 51 : 458-463. 1952.
3. Karrer, P., and E. Jucker. Carotenoids. (English edition). Elsevier Publishing Co., New York. 1950.
4. Lederer, E. Sur les carotenoids de quelques champignons. Compt. Rend. Soc. Biol. 117 : 1083-1085. 1934.
5. Lederer, E. Sur les carotenoids des cryptogrames. Bull. Soc. Chim. Biol. 20 : 611-634. 1938.
6. Newton, M., and T. Johnson. Color mutations in *Puccinia araminis tritici*. Phytopathology 17 : 711-725. 1927.
7. Newton, M., H. Johannson, and T. Johnson. A study of the carotenoid pigments of the urediospores of wheat stem rust and four of its colour variants. Phytopathology 25 : 30. 1935.

A STUDY OF SOME SEED-BORNE DISEASES OF CEREALS IN CANADA

III. EFFECT OF RATE OF SEEDING, PERCENTAGE OF INFESTED KERNELS, AND WEEDS, ON THE YIELD OF PLOTS SOWN WITH TREATED AND UNTREATED SEED[1]

J. E. MACHACEK, H. A. H. WALLACE, H. W. MEAD, AND W. C. BROADFOOT[2]

Botany and Plant Pathology Division, Science Service, Department of Agriculture, Canada

[Received for publication September 15, 1953]

ABSTRACT

Field-plot experiments with wheat seed naturally infested with *Helminthosporium sativum* P.K. & B., oat seed infested with *H. avenae* Eidam, and barley seed infested with either *H. sativum* or *H. teres* Sacc., showed that in plots kept free from weeds the percentage of infested kernels in the seed affected the percentage germination but had little or no effect on the yield. As a consequence, treatment of the seed to improve its germination also had but little or no effect on the yield. Further experiments with practically disease-free wheat seed indicated that these results were due to a compensation for low germination by an increased tillering of the plants that survived, and therefore the yield of plots with thin stands was but little different from the yield of plots with dense stands. However, when Argentine rape (*Brassica napa* L.) was sown along with the grain to simulate "weediness" in the experimental plots, the compensation for low germination by increased tillering was largely prevented, and the yield of grain from a plot was related to the number of plants in it. Under "weedy" conditions also, treatment of wheat and barley seed infested by *H. sativum*, and of oat seed infested by *H. victoriae*, increased germination and frequently the yield—the increase in yield being most apparent at low rates of seeding.

INTRODUCTION

In the first paper (8) of this series, several methods of testing wheat, oat, and barley seed for infestation with seedling-blight fungi were described and compared. In the second paper (6), an account was given of the fungi isolated from several thousand samples of wheat, oat, and barley seed collected over a five-year period from the grain-producing areas of Canada. In the present paper, an attempt is made to show the effect of seed-borne disease and seed treatment on stand and yield of wheat, oats, and barley sown at different rates in clean and weedy soil.

This investigation, commenced in 1947 and concluded in 1949, arose from an unreported, earlier (1944-1946) study of the effect on stand and yield from sowing treated and untreated cereal seed containing different percentages of infested kernels. That seed, which consisted of Regent wheat naturally infested with *Helminthosporium sativum*, Erban oats naturally infested with *H. avenae*, Charlottetown 80 barley naturally infested with *H. sativum*, and O.A.C. 21 barley naturally infested with *H. teres*, was sown in ordinary experimental plots at Winnipeg, Morden, and Brandon, in Manitoba; Saskatoon and Indian Head, in Saskatchewan; and Edmonton and Lethbridge, in Alberta. The data obtained showed that while germination decreased as the proportion of infested kernels in the seed increased, the decrease in germination was seldom followed by a

[1] Contribution No. 1309 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Senior Plant Pathologist and Asst. Plant Pathologist, Laboratory of Plant Pathology, Winnipeg, Man.; Assoc. Plant Pathologist, Laboratory of Plant Pathology, Saskatoon, Sask.; and Director, Science Service Laboratories, Lethbridge, Alta., respectively.

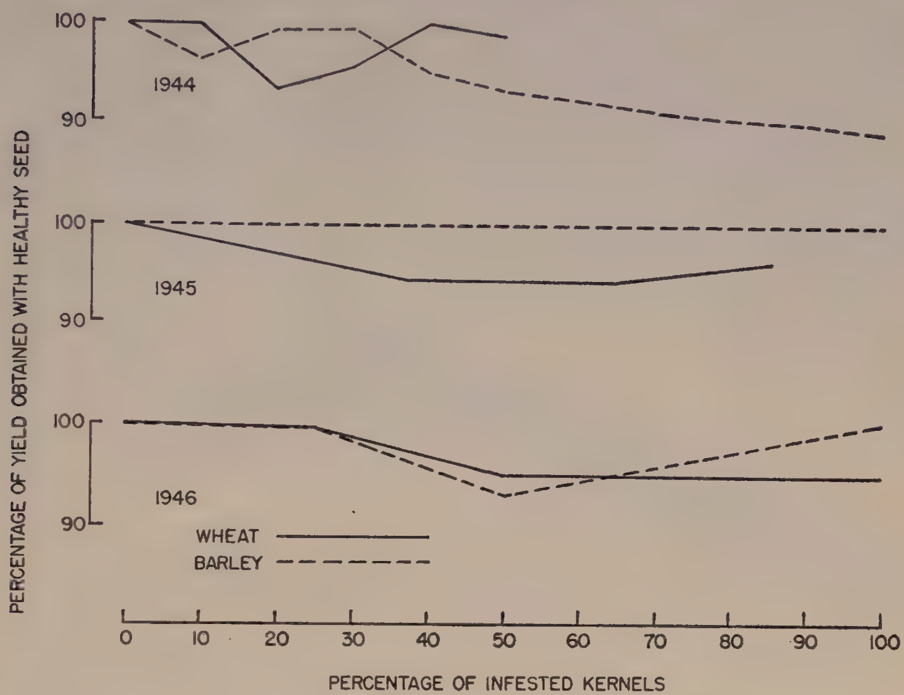


FIGURE 1. Wheat and barley seed infested with *Helminthosporium sativum:* Effect of different percentages of infested kernels in the seed on the yield of grain in weed-free experimental plots.

decrease in yield. This occurred over a period of several years, notwithstanding the fact that in some cases all the kernels in the seed sown were infested and that soil-moisture conditions during the growing period ranged from wet to dry at different stations. When a decrease in yield occurred, it was in plots sown with the most heavily infested seed. The general trend of yield for wheat and barley is shown in Figure 1.

A solution to the problem was suggested by the results of experiments made by other workers whose observations are summarized in the following section.

REVIEW OF LITERATURE

Hutchinson (3) found that wheat could be sown at rates within the range of 61 to 129 pounds per acre without any effect on yield. For oats this range was found to be 58 to 135 pounds, and for barley 44 to 116 pounds. At seeding rates below the minima given, yields were lowered. Hutchinson's findings were confirmed, in general, by the results of experiments conducted at Winnipeg in 1938 and 1939. These experiments showed (5) that in weed-free plots the ordinary rate for seeding wheat, oats, and barley could be reduced by 60 per cent without any consequent reduction in yield.

Obviously, the uniformity of yield over a wide range of seeding rates must be due to some adjustment in the yielding ability of plants. Hutchinson (3) found that for wheat sown at the rate of 129 ponds per

acre, the average number of fertile stalks per plant was only one and a half, whereas at the rate of 33 pounds, it was four. He found also that the yield of grain per head tended to increase as the seeding rate was decreased. Thayer and Rather (15) reported that tillering, length of culm, length of head, number of kernels per head, and kernel weight of barley increased as the number of plants per unit area was decreased. A similar trend for barley was noted by Sprague and Farris (14), for corn by Muhr and Rost (10), and for flax by Klages (4). The optimum rate of sowing seems to be dependent to some extent upon the variety sown (9, 10, 15), but independent of seasonal environment (15).

This adjustment in yielding ability appears to occur on a much reduced scale, or is absent altogether, when weeds are present in the crop. According to Blackman and Templeman (2), weeds prevent tillering of barley plants and reduce the size of head. If rainfall is adequate, the competition between weeds and barley is mostly for nitrogen and light, and weeds that develop early depress yields more than those that develop late. Mann and Barnes (7) found that, when the number of weeds was constant, an increase in the density of barley plants diminished the effect of weeds; and that, when the number of barley plants was constant, an increase in the number of weeds did not appreciably reduce yield until the number of weed plants exceeded the number of barley plants. Pavlychenko and Harrington (11), after observing competition between weeds and crop under conditions of low soil moisture, suggested that success under competition depends on readiness and uniformity of seed germination, on ability to develop a large assimilation surface in the early seedling stage, on possession of a large number of stomata, and on a root system with a large mass of fibre close to the soil surface but with its main roots penetrating deeply. They arranged the crops they studied in order of decreasing competitive ability, as follows: barley, rye, wheat, oats, and flax.

Cereal crops affected with root diseases seem less able to withstand competition from weeds than healthy crops. For instance, when wheat is attacked by Browning root-rot (*Pythium* spp.) its growth may be retarded and its tillering reduced to such an extent that weeds become established and presumably do further harm to the crop (1). A similar effect was observed by Russell (12) for Take-all (*Ophiobolus graminis* Sacc.), and by Sallans (13) for common root-rot (*Helminthosporium sativum*).

The experimental results mentioned above suggest, first, that sowing small grains at low rates may not unduly reduce their yield, provided that the seed used is sound and the field in which it is sown is free from weeds; and, second, that treatment of seed with the object of destroying seedling-blight fungi on it is more necessary when the soil is infested by weeds than when it is not. The reason for the latter is that an increase (due to seed treatment) in the number of plants permits the crop to withstand competition from weeds better than when the seed is not treated.

EXPERIMENTAL RESULTS

Greenhouse Experiments

Preparatory to field trials, the relation of weeds to the stand of grain sown at different rates was studied in the greenhouse. This preliminary

work was considered necessary, as the authors intended to sow Argentine rape in the field to represent "weedy" conditions and but little was known about its suitability for this purpose. Argentine rape was chosen because plenty of good seed was available for field experimentation, and because in habit of growth it resembled wild mustard (*Brassica arvensis* B.S.P.)—a weed widely distributed in Western Canada—and it was not likely to become established in the experimental field.

In the first greenhouse trial, spring wheat was sown, in quadruplicate, in 6-inch pots (each containing 4.5 pounds of black-loam soil) at rates ranging from 2 to 25 seeds per pot. When the plants matured, it was found that as the rate of seeding increased, the average number of fertile stalks per pot increased from 4.3 to 22.3, the number of fertile stalks per plant decreased from 2.1 to 0.9, the number of kernels per head decreased from 21.5 to 6.4, and the 1,000-seed weight decreased from 36.8 gm. to 27.4 gm. There was no significant difference between rates of seeding in the total yield of grain per pot.

In the second greenhouse trial, spring wheat was again sown, under the same conditions as in the first trial but with the range of seeding extended to 100 seeds per pot. It was found that as the rate of seeding rose the total number of fertile stalks per pot rose to a maximum at the 36-seed rate, the number of fertile stalks per plant declined to a minimum at the 78-seed rate, and the number of kernels per head declined to a minimum at the 40-seed rate. There was no significant change beyond the rates indicated. The total yield of grain per pot, however, rose to a maximum at the 18-seed rate, then declined to a minimum at the 74-seed rate where it levelled off. This trend in total yield per pot differed from the trend in the first trial, where the yield was the same at all rates of seeding. An interesting observation was made during the second trial. Above the 40-seed level a number of plants in some pots died from root disease, probably due to over-crowding, and the surviving plants acquired characteristics possessed by plants at lower seeding rates—more vigour, more tillering, and larger yield.

In the third greenhouse trial, the seeding rates for wheat were the same as in the first trial (2 to 25 seeds per pot), but in addition, Argentine rape was sown at the rate of 10 seeds in one-third of the pots, and in another third at 20 seeds per pot. When the wheat had headed, both wheat and rape plants were cut off at soil level and the combined green weight as well as the separate green weight was determined for each pot. These weights showed that, in all three sets of pots, the total weight of wheat increased as the seeding rate increased, and that at each seeding rate, the growth of wheat in the pots where 10 seeds of rape had been sown was less than in the pots without rape, and much less in pots where 20 seeds of rape were sown. On the other hand, the green weight of rape, in both sets of pots containing it, declined as the seeding rate for wheat increased. The total green weight (wheat and rape), except for minor deviations, was about the same in all pots.

Field Experiments

In 1947, when the first field trial with mixed cereals and "weeds" was performed, healthy Regent wheat seed was sown at seven rates, in blocks

TABLE 1.—EFFECT OF RATE OF SEEDING HEALTHY SEED ON THE NUMBER OF PLANTS AND THE YIELD OF WHEAT IN CLEAN PLOTS AND IN PLOTS INFESTED WITH ARGENTINE RAPE

Rate of seeding (lb./ac.)	Number of wheat plants per row			Yield (gm. per plot)			Yield reduction (gm.) from rape	
	No rape	Rape (5 lb./ac.)	Rape (10 lb./ac.)	No rape	Rape (5 lb./ac.)	Rape (10 lb./ac.)	Rape (5 lb./ac.)	Rape (10 lb./ac.)
10	62.6	61.6	58.3	391.0	277.5	235.0	−113.5	−156.0
20	115.0	97.6	106.3	529.0	430.5	343.0	− 98.5	−186.0
30	170.3	163.6	157.3	581.0	436.5	448.0	−144.5	−133.0
40	213.0	214.0	212.0	591.0	511.5	451.0	− 79.5	−140.0
50	292.6	222.3	256.6	588.0	516.5	498.5	− 71.5	− 89.5
60	319.0	312.0	306.3	531.5	468.0	438.0	− 63.5	− 92.5
70	372.0	351.0	341.0	480.0	485.0	430.5	+ 5.0	− 49.5
Sig. diff. (5%)	20.9	20.9	20.9	72.1	72.1	72.1		
Mean	220	203	205	527.0	446.5	406.0		
Sig. diff. (5%)	Differences not significant			—	40.6	—		

of land free from weeds and also in other blocks where Argentine rape was sown at the rates of 5 pounds and 10 pounds per acre just after the wheat. Each of the wheat plots consisted of four rows, nine inches apart, the outer rows being utilized for counts of plants and the inner two rows for yield. The rows of rape plants were also 9 inches apart, at right-angles to the rows of wheat. The data obtained from this trial showed (Table 1) that while the number of mature wheat plants was not affected by the rape, the yield was reduced, especially at low seeding rates. The yield-depressing effect of Argentine rape was more pronounced when it was sown at 10 pounds per acre than when it was sown at 5 pounds.

In the second field trial, in 1948, treated and untreated seed of Regent wheat, Garry oats, and Montcalm barley, naturally infested by seedling-blight fungi, was sown in weed-free plots and also in plots containing Argentine rape sown at 10 pounds per acre between the crop rows. In the wheat and barley seed used, all of the kernels were infested with *Helminthosporium sativum*, in the oats 69 per cent of the kernels were infested with *H. victoriae* Meehan and Murphy. The seeding rate for wheat and barley was one and a half bushels per acre, and for oats it was two bushels. Where the seed was treated, one-half ounce per bushel of 5 per cent ethyl mercury phosphate (Ceresan) was thoroughly mixed with it one day before sowing. The data from this trial, summarized in Table 2, showed that in both the weed-free plots and the ones contaminated by Argentine rape the population of mature wheat, oat, and barley plants was greater when the seed had been treated than when it was not, and that for some unknown reason the population tended to be smaller in the plots containing rape. Treatment of seed increased the yield of all three crops but the percentage increase was greater in the plots with rape than in those without it.

FIGURE 2. Seed treatment and weed control: *left*, untreated, diseased (*Helminthosporium sativum*) barley sown at 1½ bu. per acre; *middle*, untreated, diseased barley sown at ½ bu. per acre; *right*, treated, diseased barley sown at ½ bu. per acre.

The third field trial, in 1949, was a duplicate of the second field trial except that each of the three cereal crops was sown at five different rates, as indicated in Figures 3, 4, and 5. As the growing season advanced it was observed that the growth of Argentine rape was markedly affected by the seeding rate and treatment of the seed for both wheat and barley. Where untreated seed of these crops was sown at less than one bushel per acre, the rape grew well and appeared to dominate the association (Figure 2), but, when treated seed was sown at the same rate, in most plots there seemed to be enough plants of wheat or barley to withstand competition from the "weed". In oats, seed treatment increased the number of maturing plants very little, the increase being too small to influence the growth of Argentine rape. It is believed that *Helminthosporium victoriae*, which by 1949 had

TABLE 2.—EFFECT OF TREATING INFESTED SEED ON NUMBER OF PLANTS AND YIELD OF WHEAT, OATS, AND BARLEY SOWN ALONE OR ALONG WITH ARGENTINE RAPE

Data	Crop	Untreated		Treated*		Sig. diff. (5%)	Increase (%) from treatment	
		No rape	Rape	No rape	Rape		No rape	Rape
No. plants	Wheat	119.5	124.3	214.5	136.0	—	79.8	9.6
	Oats	53.5	21.8	154.3	99.0	57.2	188.4	345.1
	Barley	87.3	87.5	174.5	148.5	—	99.8	69.7
Yield (gm.)	Wheat	1384	417	1571	736	—	13.5	76.4
	Oats	629	116	840	213	282	33.5	83.6
	Barley	2056	762	2233	1235	—	8.6	62.0

* Seed treated with 5 per cent ethyl mercury phosphate at the rate of ½ oz. per bu.

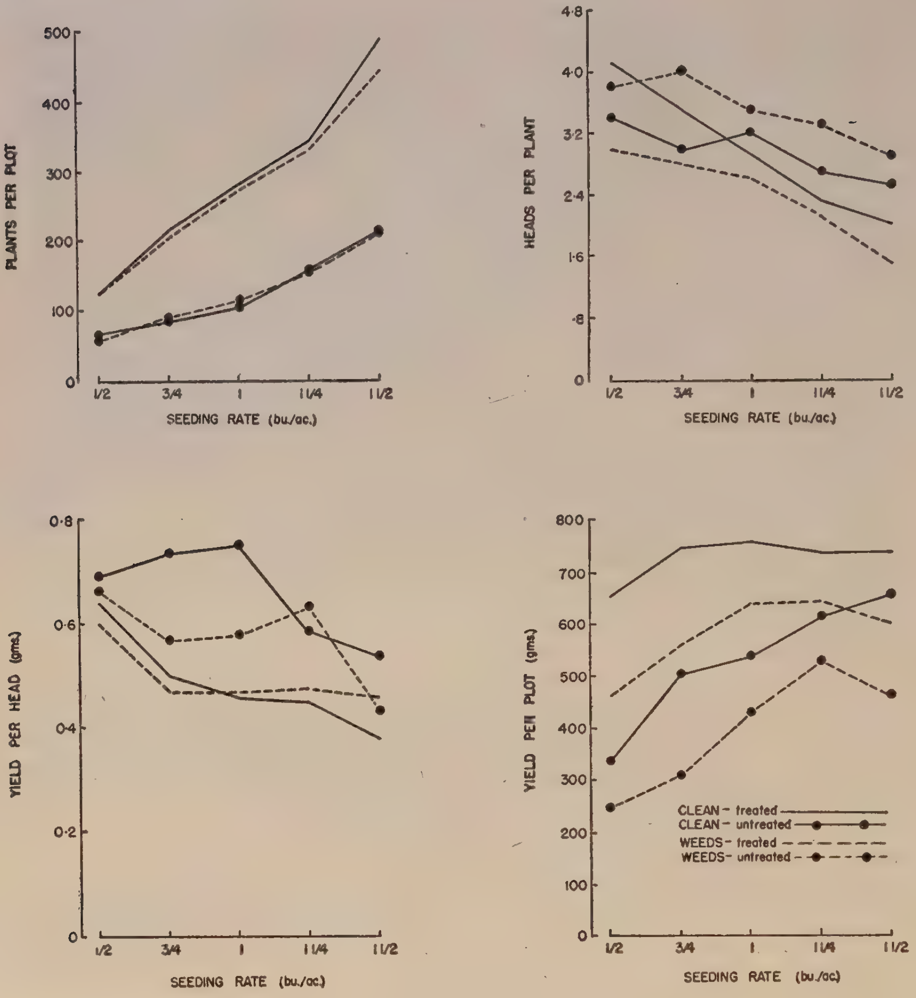


FIGURE 3. Wheat seed infested with *Helminthosporium sativum:* Effect of rate of seeding and seed treatment on number of plants per plot, number of heads per plant, yield of grain per head, and yield of grain per plot in weed-free and weedy experimental plots.

infested the experimental field very thoroughly, killed most of the additional oat seedlings arising from treated seed and thereby cancelled out the earlier gain in germination from seed treatment.

While it was noted in the second field trial that the presence of Argentine rape tended to lower the number of crop plants in a plot, this situation was not observed in the third field trial. In the second field trial also, seed treatment increased the number of plants for all three crops, while in the third trial it did not for wheat and barley only. A possible reason for the discrepancy, the soil-infestation by *H. victoriae*, has already been mentioned.

In the third field trial, the number of heads or panicles per plant was found to decrease as the seeding rate increased. As Figures 3, 4, and 5

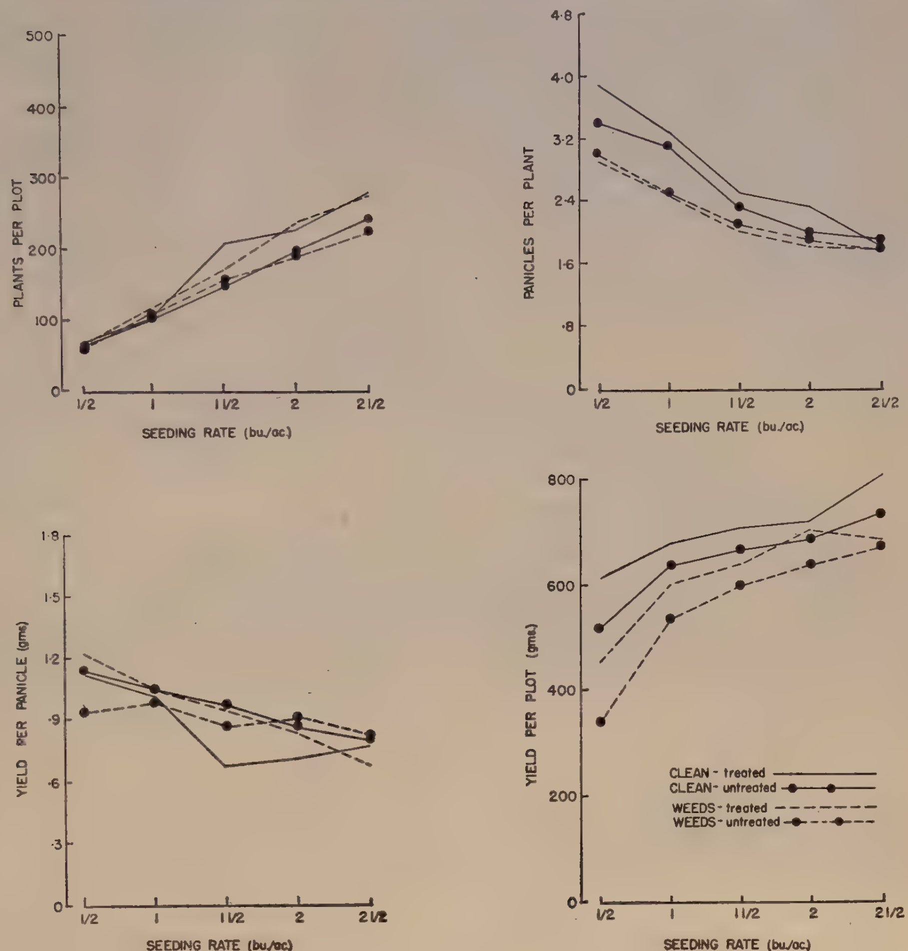


FIGURE 4. Oat seed infested with *Helminthosporium victoriae:* Effect of rate of seeding and of seed treatment on number of plants per plot, number of panicles per plant, yield of grain per panicle, and yield of grain per plot in weed-free and weedy experimental plots.

indicate, the slope of the decline was fairly uniform for wheat, but it tended to be steeper at low seeding rates than at high ones for oats and barley, especially for the latter. Seed treatment tended to lower the number of heads per plant for wheat and barley but raised it for oats. The presence of Argentine rape had a variable effect. It lowered the number of panicles and heads of oats and barley, but for wheat there seemed to be an interaction between weediness and seed treatment—the extremes in number of heads being found in weedy plots, the lowest number when the seed was sown treated and the highest number when sown untreated. In weed-free plots, the number of heads per wheat plant was higher for treated seed than for untreated seed when it was sown at a low rate but the reverse was found when the seed was sown at high rates. The trends for yield of grain per head or panicle were in some respects similar to those observed for

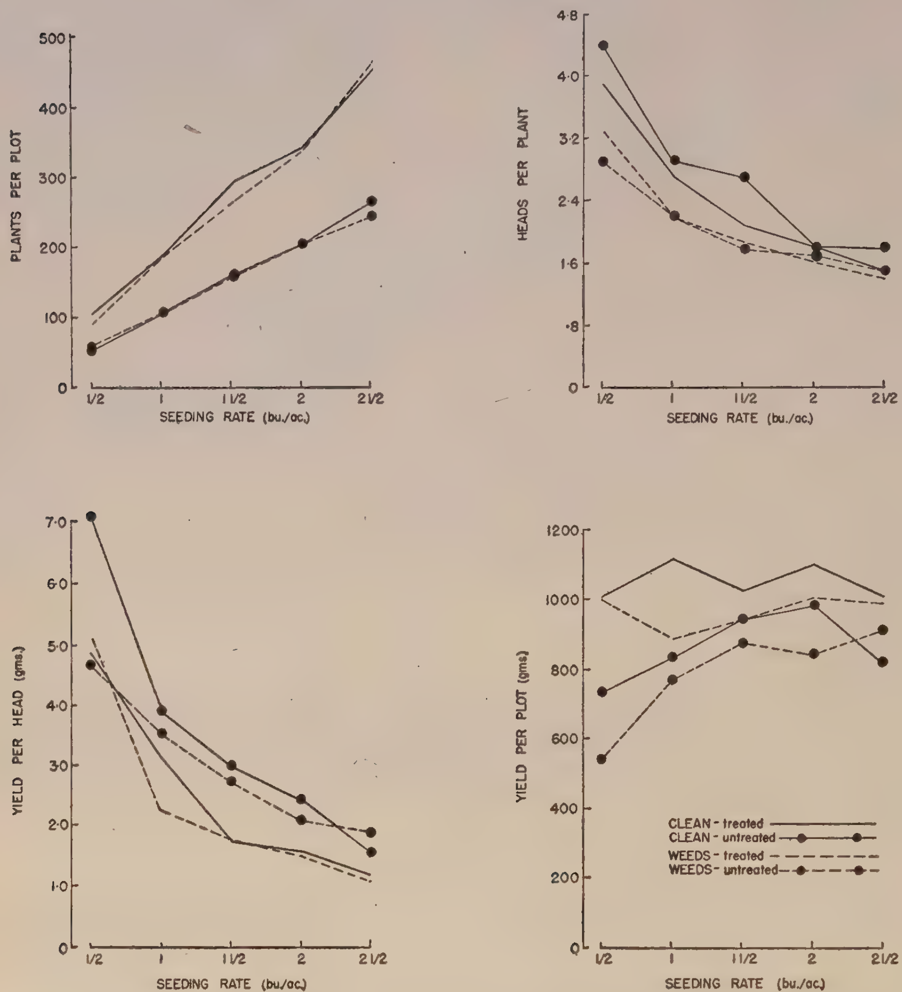


FIGURE 5. Barley seed infested with *Helminthosporium sativum:* Effect of rate of seeding and of seed treatment on number of plants per plot, number of heads per plant, yield of grain per head, and yield of grain per plot in weed-free and weedy experimental plots.

number of heads or panicles per plant. In general, the yield per head decreased as the seeding rate increased, treatment of the seed lowered the yield for wheat and barley but not for oats, and the presence of Argentine rape had a variable effect. Yield of grain per plot, for all three crops, tended to increase as the seeding rate was increased but there were some differences between crops. For wheat, when treated seed was sown in weed-free plots a gain in yield was obtained only when the seeding rate was raised from one-half to three-quarters of a bushel per acre. When treated seed was sown in "weedy" plots, the increase in yield held for two consecutive increases in seeding rate ($\frac{1}{2}$–$\frac{3}{4}$ bu./ac. and $\frac{3}{4}$–1 bu./ac.). Increases in yield for three consecutive increases in seeding rates occurred when untreated seed was

TABLE 3.—CORRELATION BETWEEN PAIRS OF FACTORS RELATING TO YIELD IN UNTREATED AND TREATED DISEASED WHEAT SEED SOWN IN CLEAN AND "WEEDY" PLOTS

Correlation between	Coefficient*			
	No rape		Rape	
	Untreated seed	Treated seed	Untreated seed	Treated seed
Number of plants per lot and number of heads per plant	− *0.834*	− *0.907*	− *0.714*	− *0.779*
Number of plants per plot and yield per head	− *0.759*	− *0.868*	− *0.576*	− 0.389
Number of plants per plot and yield per plot	+ *0.809*	+ *0.491*	+ *0.799*	+ *0.561*
Number of heads per plant and yield per head	+ 0.076	+ *0.709*	+ 0.309	− 0.005
Number of heads per plant and yield per plot	− *0.656*	− 0.414	− *0.498*	− 0.110
Yield per head and yield per plot	− 0.413	− *0.723*	− 0.109	− 0.123

* The coefficients shown in italics type are statistically significant at the 5 per cent level.

sown in weedy plots, and for four consecutive increases in seeding rate when untreated seed was sown in weed-free plots. For oats, each increase in seeding rate resulted in an increase in yield, irrespective of the conditions under which the seed was sown except in one instance—when treated seed was sown in plots containing Argentine rape and the seeding rate was raised from two to two and a half bushels per acre. For barley, the yields per plot were all at about the same level when treated seed was sown in weed-free plots, and again at about the same but lower level when sown in weedy plots. When the seed was not treated, increases in the seeding rate resulted in increases in yield, except at rates above one and a half bushels per acre. In general, higher yields were obtained from treated seed and clean soil than from untreated seed and weedy soil.

To secure additional information regarding the factors affecting yield, the data from the wheat part of the third field trial were correlated. The coefficients of correlation obtained are given in Table 3.

DISCUSSION

From the foregoing, it would appear that when seed of cereal crops is heavily infested with seedling-blight fungi but is free or nearly free from smut and is sown in weed-free soil, its treatment with fungicides is of but limited value. The chief reasons for this situation seem to be as follows: first, when untreated, infested seed is sown, a considerable proportion of the infested seeds germinate and the seedlings from them either fail to become infected or recover from the attack; and second, in weed-free soil the seedlings that survive tiller more and produce larger heads or panicles

than when treated seed is sown under the same conditions. The result is that, unless the seed is infested by a particularly virulent pathogen that reduces germination greatly, the yield from untreated, infested seed approaches or equals the yield obtained from uninfested or treated, infested seed.

However, the land on which cereals are grown is not always clean but is infested in various degrees with weeds. The competition from aggressive weeds reduces the productivity of crop plants by reducing their growth and tillering. Under these conditions, when the seed is severely infested by seedling blight fungi seed treatment is necessary, as the resulting increase in germination provides a growth dense enough to withstand competition from weeds. A similar result may be obtained, of course, by increasing the rate of seeding for the crop.

A survey of cereal seed produced in Canada has shown that while moderate to severe infestation by seedling blight fungi is common for seed produced in Eastern Canada, an equivalent, but mechanical, injury occurs in seed produced in Western Canada. The latter injury consists of an exposure of the seed embryo or endosperm, and when such seed is sown, invasion by soil micro-organisms may kill the seed or greatly weaken the seedling therefrom. Treatment of mechanically-injured seed prevents such invasion and most of the seeds germinate normally. As both kinds of injury often occur in the same lot of seed, the need for treating it increases, particularly when it is to be sown in fields where weeds are likely to be abundant.

The widespread use of herbicides in grain-growing areas has probably modified to some extent the need for seed treatment. If a herbicide is to be applied to a field, treatment of the seed with a fungicide may not be necessary, especially when the seed is known to be free from smut. Weedy fields, if sprayed with suitable herbicides early enough, may be considered to be equivalent to weed-free fields, and for the latter it has been shown that, unless the infestation of the seed is very severe or unless uninfested seed is sown at a low rate, the increase in growth of individual plants generally offsets a considerable reduction in their population.

Finally, in addition to the accepted recommendations respecting seed treatment for cereals, i.e., to treat with approved fungicides all seed of which the health status is unknown, to treat all seed contaminated by smut, and to treat all seed that is severely injured mechanically, a further recommendation is suggested, namely, to treat all seed that is heavily infested by seedling blight fungi when it is likely to be sown in fields infested by weeds (especially when the use of herbicides is not contemplated).

REFERENCES

1. Anonymous. Root rots of cereals. Circular No. 72, Dom. Expt. Farms, Ottawa, Canada. 1929.
2. Blackman, G. E., and W. G. Templeman. The nature of the competition between cereal crops and annual weeds. J. Agr. Sci. 28 : 247-271. 1938.
3. Hutchinson, R. E. Rates of seeding wheat and other cereals with irrigation. J. Amer. Soc. Agron. 28 : 699-703. 1936.
4. Klages, K. H. Spacing in relation to the development of the flax plant. J. Amer. Soc. Agron. 24 : 1-17. 1932.
5. Machacek, J. E., and F. J. Greaney. Further experiments on the control by seed disinfection of root-rotting fungi in wheat. Phytopath. 31 : 379-394. 1941.

6. Machacek, J. E., W. J. Cherewick, H. W. Mead, and W. C. Broadfoot. A study of some seed-borne diseases of cereals in Canada. II. Kinds of fungi and prevalence of disease in cereal seed. Sci. Agr. 31 : 193-206. 1951.

7. Mann, H. H., and T. W. Barnes. The competition between barley and certain weeds under controlled conditions. Ann. Appl. Biol. 32 : 15-22. 1945.

8. Mead, H. W., W. C. Broadfoot, and J. E. Machacek. A study of some seed-borne diseases of cereals in Canada. I. Comparison of seed testing methods. Sci. Agr. 30 : 350-360. 1950.

9. Mooers, C. A. Planting rates and spacing for corn under southern conditions. J. Amer. Soc. Agron. 12 : 1-22. 1920.

10. Muhr, G. R., and C. O. Rost. The effect of population and fertility on yields of sweet corn and field corn. Agron. J. 43 : 315-319. 1951.

11. Pavlychenko, T. K., and J. B. Harrington. Competitive efficiency of weeds and cereal crops. Can. J. Res. 10 : 77-94. 1934.

12. Russell, R. C. Studies in cereal diseases. X. Studies of take-all and its causal organism, *Ophiobolus graminis* Sacc. Canada Dept. Agr. Bull. 170. New series. 1934.

13. Sallans, B. J. The relationship of weeds to losses caused by common rootrot in wheat. Sci. Agr. 20 : 632-637. 1940.

14. Sprague, H. B., and N. F. Farris. The effect of uniformity of spacing seed on the development and yield of barley. J. Amer. Soc. Agron. 23 : 516-533. 1931.

15. Thayer, J. W., and H. C. Rather. The influence of rate of seeding upon certain plant characters in barley. J. Amer. Soc. Agron. 29 : 754-760. 1937.

MINERAL AND PROTEIN CONTENT OF FORAGE CROPS IN CENTRAL SASKATCHEWAN[1]

J. M. Bell,[2] P. D. McLaren and Greta McKay[3]

University of Saskatchewan, Saskatoon, Saskatchewan

[Received for publication October 2, 1953]

ABSTRACT

Chemical determinations for total protein, total ash, crude silica, calcium, phosphorus, magnesium, potassium, sodium and manganese are reported for grass and legume hays common to this area. The species included 14 cultivated grasses, 4 legumes, 12 native upland grasses including some weeds with potential feeding value, 7 native lowland grasses and sedges and 10 grasses and legumes grown on alkali soils.

The data are discussed in relation to nutrient requirements of cattle. Protein contents ranged from 5.2 to 19.5 per cent of the dry matter. Mineral contents were: calcium 0.08 to 0.89 per cent, phosphorus 0.03 to 0.46 per cent, magnesium 0.1 to 0.8 per cent, potassium 0.18 to 3.73 per cent, sodium 0.005 to 0.384 per cent and manganese 12 to 317 p.p.m. A general need for supplemental protein, calcium, phosphorus and salt was indicated for grass hays but not so for legumes. The minerals, magnesium, potassium and manganese, do not appear to present problems in this area.

INTRODUCTION

Minerals and protein are often major limiting factors in ruminant feeds, particularly in regions where legumes are not grown extensively. Under such conditions it is not unusual to find digestible crude protein (D.C.P.) contents of cured grasses as low as 3 to 6 per cent (2), well below the level required by growing or producing cattle. It is possible that similar conditions may prevail with respect to minerals. Orr has stated in a foreword to Russell's (15) review on minerals: "If the pasture contains all the inorganic nutrients needed for health and is sufficient in quantity, there is unlikely to be a deficiency of either vitamins or protein".

The deficiency syndromes of phosphorus, calcium, salt, iodine and cobalt are now well known but evidence regarding the roles of the other essential elements in ruminant nutrition is limited. Several investigators have demonstrated important relationships between minerals and the processes of digestion (4, 5, 7, 8).

Although the importance of proper mineral nutrition is apparent, little is known about the actual mineral content of forages, with the exceptions of calcium and phosphorus and of certain minerals in acute problem areas. Vegetation in Western Canada is frequently deficient in phosphorus, occasionally in calcium (6), and may sometimes contain inadequate cobalt (3). No data exist for the other mineral elements in forages grown in this region but in view of the wide range of soil types, moisture conditions and plant species involved, such information would assist materially in making recommendations for mineral feeding.

This report contains data on calcium, phosphorus, magnesium, sodium, potassium, manganese, crude silica and protein for the grasses and legumes common to central Saskatchewan, and for certain samples collected from alkali areas.

[1] Contribution from the Department of Animal Husbandry.
[2] Professor of Animal Husbandry.
[3] Student assistants.

TABLE 1.—DESCRIPTION OF CULTIVATED GRASSES AND LEGUMES SAMPLED AT RECOMMENDED STAGE OF MATURITY FOR HARVESTING AS HAY

Common name	Botanical name	Date harvested	Height (inches)	Remarks
Russian wild rye	*Elymus junceus* Fisch.	12/6/52	28	Thick stand, leafy
Kentucky blue grass	*Poa pratensis* L.	17/6/52	10	Good stand, leafy
Fairway crested wheat grass	*Agropyron cristatum* (L.) Gaertn.	17/6/52	18	Good stand, leafy
Brome grass	*Bromus inermis* Leyss.	17/6/52	24	Good stand, leafy
Standard crested wheat grass	*Agropyron desertorum* (Fisch.) Schult.	17/6/52	22	Good stand, leafy
Slender wheat grass	*Agropyron trachycaulum* (Link) Malte	17/6/52	20	Good stand, very leafy
Creeping red fescue	*Festuca rubra* L.	20/6/52	14	In rows, leafy
Macoun's wild rye*	*Elymus macounii* Vasey	20/6/52	30	In rows, moderately leafy
Timothy	*Phleum pretense* L.	27/6/52	24	In rows, moderately leafy
Spring rye	*Secale cereale* L.	30/6/52	30	Good stand in field
Tall wheat grass	*Agropyron elongatum* (Host) P.B.	9/7/52	36	In rows, leafy
Intermediate wheat grass	*Agropyron intermedium* (Host) P.B.	9/7/52	34	In rows, leafy
Oat hay	*Avena sativa* L.	14/7/52	34	Good stand in field
Reed canary grass	*Phalaris arundinacea* L.	23/6/52	26	Thick stand, quite leafy
Russian wild rye	*Elymus junceus* Fisch.	12/6/52	6	In rows, quite leafy. Alkali soil
Brome grass	*Bromus inermis* Leyss.	17/6/52	12	Thin stand, mostly leaves. Alkali soil
Slender wheat grass	*Agropyron trachycaulum* (Link) Malte	17/6/52	12	Thick stand, mostly leaves. Alkali soil
Tall wheat grass	*Agropyron elongatum* (Host) P.B.	12/7/52	26	In rows, very leafy. Alkali soil
"Quill Lake No. 1"	Mixed grasses	1951	15	June grass, Western rye grass, rough fescue. Alkali soil
"Quill Lake No. 2"	Mixed grasses	1951	14	June grass, Western rye grass. Alkali soil
"Quill Lake No. 3"	Mixed grasses	1951	26	Brome grass, wild barley. Alkali soil
Crested wheat grass	*Agropyron cristatum* (L.) Gaertn.	1951	?	Mostly crested wheat grass. Alkali soil
Yellow sweet clover	*Melilotus officinalis* (L.) Lam.	19/6/52	42	Thick, leafy stand.
White sweet clover	*Melilotus alba* Desv.	23/6/52	45	In rows, moderately leafy
Alfalfa	*Medicago sativa* L.	27/6/52	14	Good stand, leafy
Alsike clover	*Trifolium hybridum* L.	2/6/52	12	Taken from mixed sward
Yellow sweet clover	*Melilotus officinalis* (L.) Lam.	17/6/52	28	Good stand. Alkali soil
Kochia	*Kochia scoparia* (L.) Schrad.	26/7/52	48	Thick stand, 6-inch rows

* Actually a native grass grown under cultivation on an experimental basis.

TABLE 2.—DESCRIPTION OF NATIVE PLANTS SAMPLED AT RECOMMENDED STAGE OF MATURITY FOR HARVESTING AS HAY

Common name	Botanical name	Date* harvested	Height (inches)	Remarks
June grass	*Koeleria cristata* (L) Pers	12/6/52	6	Thin, stand leafy
Spear grass	*Stipa comata* Trin. and Rupr.	21/6/52	12	Good stand
Rough fescue	*Festuca scabrella* Torr.	27/6/52	10	Leafy, in mixed sward
Russian thistle	*Salsola pestifer* A. Nels.	30/6/52	12	Thick stand, leafy
Western wheat grass	*Agropyron smithii* Rydb.	2/7/52	18	Thick stand, leafy
Couch grass	*Agropyron repens* (L.) Beaur.	2/7/52	27	Thick stand, leafy
Green needle grass	*Stipa viridula* Trin.	2/7/52	27	Mixed sward, leafy
Indian rice grass	*Oryzopsis hymenoides* (Roem. and Schalt.) Ricker	7/7/52	26	Thick, leafy stand
Sand grass	*Calamovilfa longifolia* (Hook.) Scribn.	7/7/52	27	Thin leafy stand
Sand dropseed	*Sporobolus cryptandrus* (Torr.) A. Gray	7/7/52	20	Very thin stand, leafy
Canada wild rye	*Elymus canadensis* L.	7/7/52	30	Very thin stand, leafy
Salt grass	*Distichlis stricta* (Torr.) Rydb.	17/6/52	4	Thick, leafy stand. Alkali soil
Wild barley	*Hordeum jubatum* L.	23/6/52	14	Thin, leafy stand
Wild barley	*Hordeum jubatum* L.	23/6/52	12	Thin, leafy stand. Alkali soil
Alkali grass	*Puccinella nuttalliana* (Schultes) Hitche	23/6/52	18	Thin stand, moderately leafy. Alkali soil
Wire grass	*Juncus ater* Rydb.	30/6/52	18	Thick stand
Slough grass	*Bechmannia syzigachne* (Steud.) Fernald	2/7/52	24	Fair stand, moderately leafy
Lowland grass	*Carex* spp.	2/7/52	28	Thick stand, very leafy
Spangle top	*Fluminea festucacea* (Willd.) Hitche	30/6/52	54	Thick stand, leafy

* Day/month/year.

MATERIALS AND METHODS

Collection of Samples

The plants collected for analysis were taken during the early bloom stage so as to conform as nearly as possible to recommended haying practices. Care was taken to obtain only pure-species samples and to avoid undue contamination from soil. The only exceptions to this were the samples submitted from the Quill Lake region in which area mineral feeding problems had arisen. The samples were typical of the pastures in question.

Analyses

Crude protein, moisture and total ash determinations were made on all samples in the usual manner. For calcium, phosphorus, magnesium, manganese, sodium, crude silica and potassium, duplicate five gram samples were 'wet' ashed with $HClO_4$, HNO_3 and H_2SO_4 (12). Phosphorus was determined colorimetrically with a Beckman DU spectrophotometer according to Barton's method (1); manganese was also assayed colorimetrically (1, 17) and crude silica represented the residue removed by filtration from the sample, following digestion and dilution to 100 ml. The remainder of the elements were determined by flame spectrophotometry(11).

RESULTS AND DISCUSSION

Identifications and descriptions of materials studied are given in Tables 1 and 2. Most of the cultivated grasses and legumes were obtained from the experimental plots of the Dominion Forage Crops Laboratory at Saskatoon but some were taken from the University Farm or from alkali test plots in the vicinity of Saskatoon. In most instances comments are provided as to height and appearance of the stand of forage at time of sampling, but it would be well to recall that moisture conditions during the year (1952) were unusually good and consequently the growth of the grasses may have been better than average.

The chemical compositions of the various forages are presented in Table 3.

Calcium and Phosphorus

In the case of the cultivated grasses calcium contents deviated but little from 0.2 per cent; exceptions being Macoun's wild rye at 0.08 per cent and creeping red fescue at 0.35 per cent. When these levels are compared with the recommended allowances (13, 14) it would appear that the amount of calcium in the cultivated forages studied would be borderline or insufficient to meet the needs of cattle under about one year of age and of breeding and nursing females. With a few exceptions the same would apply to phosphorus since the contents ranged from 0.06 to 0.25 per cent compared to recommended levels of up to 0.28 per cent for young stock.

The legume hays contained abundant calcium and slightly more phosphorus than did the cultivated grasses although perhaps still insufficient to meet the needs of rapidly growing cattle.

The native upland grasses had calcium contents varying from 0.15 to 0.34 per cent and phosphorus levels of 0.15 to 0.38 per cent. In general the native species differed but little from cultivated grasses although a number of them did appear to contain enough phosphorus to obviate the need for a phosphorus feed-supplement for cattle; June grass, spear grass, Canada wild rye, western wheat grass and sand dropseed contained 0.27 per cent phosphorus or more and fall readily within such a category. Russian thistle (10), while not a grass, contains rather large amounts of both elements.

TABLE 3.—MINERAL AND PROTEIN CONTENT OF GRASSES AND LEGUMES AT SASKATOON (ALL VALUES REPORTED ON A MOISTURE-FREE BASIS)

Forage	Protein	Ash	Crude silica	Ca	P	Mg	K	Na	Mn
	%	%	%	%	%	%	%	%	p.p.m.
Cultivated grasses—									
Brome grass	14.6	6.19	2.3	0.21	0.17	0.3	0.88	0.006	44
Oat hay	13.1	6.70	1.4	0.18	0.22	0.1	1.11	0.167	24
Russian wild rye	13.0	6.97	2.4	0.19	0.15	0.1	0.90	0.015	34
Standard crested wheat grass	12.8	5.47	2.4	0.17	0.18	0.2	0.69	0.011	38
Macoun's wild rye*	12.8	5.23	1.9	0.08	0.09	0.1	0.94	0.014	25
Slender wheat grass	12.6	6.63	3.8	0.20	0.19	0.1	0.57	0.009	45
Fairway crested wheat grass	12.3	5.87	2.7	0.23	0.17	0.1	0.68	0.009	44
Creeping red fescue	11.8	7.30	3.4	0.35	0.14	0.1	0.77	0.010	45
Reed canary grass	11.6	9.56	4.1	0.22	0.25	0.1	1.01	0.010	61
Kentucky blue grass	8.6	6.71	3.5	0.23	0.25	0.2	0.52	0.009	36
Tall wheat grass	8.3	7.15	3.5	0.20	0.11	0.2	0.68	0.173	35
Spring rye	6.7	5.30	2.1	0.17	0.23	0.1	0.46	0.005	29
Intermediate wheat grass	6.4	6.74	4.7	0.24	0.06	<0.1	0.53	0.010	49
Timothy	5.3	5.24	2.2	0.21	0.15	0.3	0.61	0.009	26
Cultivated legumes—									
Alfalfa	19.0	7.57	2.7	0.82	0.23	0.2	0.81	0.076	23
Yellow sweet clover	16.4	6.62	0.2	0.48	0.24	0.1	1.02	0.010	16
Alsike clover	16.0	7.55	3.4	0.67	0.27	0.3	1.15	0.009	33
White sweet clover	12.6	5.79	0.2	0.56	0.23	0.2	0.79	0.008	15
Native upland 'grasses'—									
Russian thistle	19.5	21.97	7.9	0.89	0.46	0.7	3.73	0.037	62
Kochia	13.0	10.68	1.6	0.67	0.22	0.8	2.27	0.384	39
June grass	11.3	5.80	3.4	0.21	0.27	0.2	0.51	0.006	33
Spear grass	10.8	7.34	4.1	0.22	0.32	0.1	0.59	0.009	50
Canada wild rye	9.3	6.98	3.6	0.30	0.30	0.2	0.64	0.011	23
Western wheat grass	9.0	7.24	4.1	0.26	0.38	0.2	0.82	0.006	24
Rough fescue	8.1	5.38	3.4	0.18	0.19	0.2	0.38	0.010	22
Sand dropseed	7.7	6.63	3.1	0.21	0.27	<0.1	0.65	0.008	25
Indian rice grass	7.4	6.17	3.3	0.34	0.21	0.1	0.54	0.007	32
Couch grass	7.3	6.98	4.6	0.15	0.15	0.2	0.87	0.041	78
Sand grass	7.2	4.85	2.3	0.30	0.21	<0.1	0.63	0.008	24
Green needle grass	6.0	6.25	4.1	0.25	0.21	<0.1	0.53	0.007	42
Native lowland grasses and sedges—									
Slough grass	15.8	5.66	3.5	0.17	0.21	<0.1	0.72	0.010	44
Salt grass	11.7	7.07	3.5	0.15	0.22	0.2	0.56	0.103	33
Wire grass	9.2	14.48	6.8	0.61	0.29	0.3	0.83	0.126	232
Lowland sedge	7.7	10.02	5.6	0.33	0.26	0.2	0.92	0.011	317
Spangle top	7.2	6.70	4.0	0.18	0.25	0.3	0.66	0.055	218
Wild barley	6.2	5.93	3.2	0.23	0.26	0.1	0.60	0.009	29
Alkali grass	6.2	5.49	2.6	0.15	0.25	0.1	0.62	0.043	26

* See footnote, Table 1.

Among the native species inhabiting lowland areas only wire grass and lowland sedge contained sufficient calcium for young growing cattle and these do not rank high as hay crops in other respects, particularly in palatability. The others had calcium contents resembling those of cultivated grasses. Phosphorus levels slightly exceeded those of most cultivated grasses.

Magnesium

So far as magnesium is concerned, most of the forages tested contained between 0.1 and 0.3 per cent of the element. Huffman *et al.* (9, 13) have reported the magnesium requirement for dairy calves as being about 0.6 grams per 100 pounds liveweight daily which indicates that something less than 0.1 per cent of available magnesium in the feed would suffice.

Potassium

The potassium levels fell largely within the range 0.5 to 0.8 per cent, notable exceptions being Russian thistle and kochia with values of 3.73 and 2.27 per cent respectively. Likewise the total ash and magnesium values were high in these forages and in both instances the feeds have tendencies to be laxative in nature. The possibility thus exists that these two plants would contain excessive amounts of certain elements if they constituted a major part of the ration but in general there is no indication that potassium presents a problem in ruminant feeding in this area.

Sodium

In studying the salt needs of cattle Theiler *et al.* (16) found the sodium requirements to be about 1.5 grams per day for growth. Thus cattle consuming two pounds of dry matter daily per 100 pounds liveweight would have their sodium requirements met if the forage contained 0.03 per cent sodium. Of the samples studied only oat hay, tall wheat grass, alfalfa, Russian thistle, kochia, couch grass, salt grass, wire grass, spangle top and alkali grass would have supplied this amount. Even with these feeds however it is doubtful if the need for supplementary salt would be appreciably lowered because salt has some value as a condiment, quite aside from supplying the elements sodium and chlorine.

Manganese

Relatively little research has been done on the manganese requirements of farm livestock. It would seem obvious however, if the manganese requirement is in the vicinity of 6 parts per million of feed (13), that there is little likelihood of a deficiency occurring in this area. None of the forages contained less than 15 p.p.m. Three lowland plants, wire grass, lowland sedge and spangle top, had 200 to 300 p.p.m. and since they did not all grow in the same location it is improbable that the high manganese levels were due to peculiar soil composition.

Protein

In discussing the protein contents of the forages it will be noted that the protein values ranged from 5.3 to 19.5 per cent. The majority of the cultivated grasses, unlike the native ones, contained over 11.5 per

TABLE 4.—MINERAL AND PROTEIN CONTENT OF FORAGES GROWN ON ALKALINE AND NON-ALKALINE SOILS (ALL VALUES REPORTED ON MOISTURE FREE BASIS)

Forage	Soil type	Height	Protein	Ash	Crude silica	Ca	P	Mg	K	Na	Mn
		in.	%	%	%	%	%	%	%	%	p.p.m.
Russian wild rye	Non-alkali	28	13.0	6.97	2.4	0.19	0.15	0.1	0.90	0.015	34
	Alkali	6	9.2	9.55	4.5	0.22	0.19	0.2	0.58	0.126	22
Brome grass	Non-alkali	24	14.6	6.19	2.3	0.21	0.17	0.3	0.88	0.006	44
	Alkali	12	8.6	8.92	4.7	0.23	0.20	0.3	0.79	0.009	17
Slender wheat grass	Non-alkali	20	12.6	6.63	3.8	0.20	0.19	0.1	0.57	0.009	45
	Alkali	12	7.1	6.93	4.1	0.23	0.21	0.1	0.63	0.010	25
Tall wheat grass	Non-alkali	36	8.3	7.15	3.5	0.20	0.11	0.2	0.68	0.173	35
	Alkali	26	6.1	8.36	4.8	0.20	0.12	0.1	0.64	0.196	27
Wild barley	Non-alkali	14	6.2	5.93	3.2	0.23	0.26	0.1	0.60	0.009	29
	Alkali	12	5.2	8.51	5.3	0.16	0.28	0.1	0.68	0.046	30
Average	Non-alkali	24	10.9	6.57	3.0	0.21	0.18	0.2	0.73	0.042	37
	Alkali	14	7.2	8.45	4.7	0.21	0.20	0.2	0.66	0.077	24
Yellow sweet clover	Non-alkali	42	16.4	6.62	0.2	0.48	0.24	0.1	1.02	0.010	16
	Alkali	28	16.7	8.45	0.2	0.43	0.16	0.4	1.02	0.038	12
Crested wheat grass	Non-alkali	18	12.3	5.87	2.7	0.23	0.17	0.1	0.68	0.009	44
	Alkali*	—	5.3	10.50	9.1	0.36	0.07	0.1	0.26	0.005	99
Quill Lake No. 1	Alkali	15	8.3	8.76	6.6	0.30	0.03	0.1	0.33	0.047	24
No. 2	Alkali	14	8.6	9.44	7.6	0.27	0.05	0.1	0.18	0.063	32
No. 3	Alkali	25	9.0	8.65	6.6	0.31	0.15	0.4	0.59	0.016	84

* This and the following samples were obtained from the Quill Lake region in east central Saskatchewan.

cent protein as sampled and cured under ideal haying conditions. Tall wheat grass and intermediate wheat grass contained less protein than usual according to Forage Crops Laboratory records. Nevertheless it is of interest to compare the protein contents of the forages with the recommended nutrient allowances for cattle of various classes (13, 14). Comparisons indicate that about half the grasses studied do not contain sufficient protein to meet the recommendations for lactating or young growing cattle.

Effects of Alkaline Soils on Forage Composition

Comparisons are given in Table 4 for four cultivated grasses, one native grass and one legume grown on alkaline and non-alkaline soils in the Saskatoon area. While it is true that soil differences in respects other than 'alkali' content may have occurred, the effects on plant growth and composition appear to have been quite consistent. The table also contains analyses for a number of forages obtained from outlying areas in which nutritional problems in cattle had arisen.

The presence of alkali definitely restricted the growth of grasses and caused a simultaneous one-third decrease in amount of protein. Increased contents of total ash, crude silica and sodium also resulted from alkali soils. Calcium, phosphorus and magnesium appear to have been affected very little whereas decreases were noted in potassium and manganese. Yellow sweet clover, a plant recognized as having a degree of alkali tolerance, suffered some inhibition in growth but no real change in protein or in crude silica as occurred in the grasses. The effects on phosphorus, magnesium and potassium also differ somewhat from the effects in non-legumes.

The last four 'alkali' samples listed in Table 4 were either crested wheat grass or mixed species of grasses from pastures in the Quill Lake region in east central Saskatchewan. Cattle on these pastures were unthrifty and some death losses had occurred but after the provision of bone meal and cobaltized salt improvement was noted. The chemical analyses of the forages showed high values for ash and crude silica. Calcium and magnesium contents appeared to be 'normal' but in three instances the phosphorus values were exceptionally low. The problem thus appears to have been one of acute phosphorus deficiency although the possibility of cobalt involvement remains to be explored.

CONCLUSIONS

In general the forage analyses here reported indicate needs for supplemental protein, calcium, phosphorus and common salt when grass hays (cultivated or native) are fed to milking or growing cattle but there appears to be no need for additional magnesium, potassium or manganese. It should be emphasized however that season and stage of maturity affect plant composition and that the values reported probably would not apply too well to grazing conditions, or to forages cured under less favourable conditions. Legume crops in this survey differed from the non-legumes mainly in protein and calcium contents, both of which were higher in legumes. Phosphorus contents exceeded 0.2 per cent so it is likely that

extensive use of legumes in forage crops would substantially reduce or eliminate the need for supplemental minerals other than salt, iodine and possibly cobalt.

When grasses grown on alkali soils are fed the need for supplemental protein appears to become more acute although the effect on sweet clover may be less clear. Possibly local variations in soil fertility, in addition to alkalinity, account for acute deficiencies arising in some regions.

ACKNOWLEDGMENTS

Financial assistance was generously provided by the Pioneer Grain Company Limited, Winnipeg, sponsor of the Pioneer Agricultural Research Fund, and by the Saskatchewan Research Council. The authors are especially indebted to W. J. White, J. L. Bolton and R. P. Knowles, of the Dominion Forage Crops Laboratory, Saskatoon, for assistance in the collection and identification of plants and for reviewing the manuscript. The assistance given by A. Ballantyne, Experimental Farms Service, Saskatchewan Soil Survey, in doing the flame spectrophotometry and by T. C. Bunn in doing the protein analyses is also deeply appreciated.

REFERENCES

1. Barton, C. J. Photometric analysis of phosphate rock. Anal. Chem. 20 : 1068-1072. 1948.
2. Bell, J. M., G. H. Bowman, and R. T. Coupland. Chemical composition and digestibility of forage crops grown in central Saskatchewan, with observations on kochia species. Sci. Agr. 32 : 463-473. 1952.
3. Bowstead, J. E., J. P. Sackville, and R. D. Sinclair. The development of cobalt deficiency in sheep. Sci. Agr. 22 : 314-325. 1942.
4. Burroughs, W., H. G. Headley, R. M. Bethke, and P. Gerlaugh. Cellulose digestion in good and poor quality roughages using an artificial rumen. J. Anim. Sci. 9 : 513-522. 1950.
5. Burroughs, W., A. Latona, P. DePaul, P. Gerlaugh, and R. M. Bethke Mineral influences upon urea utilization and cellulose digestion by rumen microorganisms using the artificial rumen technique. J. Anim. Sci. 10 : 693-705. 1951.
6. Clarke, S. E., and E. W. Tisdale. The chemical composition of native forage plants of southern Alberta and Saskatchewan in relation to grazing practices. Can. Dept. Agr. Public. No. 769 (Tech. Bull. No. 54). 1945.
7. Gall, L. S., S. E. Smith, D. E. Becker, C. N. Stark, and J. K. Loosli. Rumen bacteria in cobalt deficient sheep. Science 109 : 468-469. 1949.
8. Hoflund, S., and H. Hedstrom. The connection between deficiency diseases and disturbances in rumen digestion. Acta Agric. Suecana 3 : 121-134. 1949.
9. Huffman, C. F., C. L. Conley, C. C. Lightfoot, and C. W. Duncan. Magnesium studies in calves. II. The effect of magnesium salts and various natural feeds upon the magnesium content of the blood plasma. J. Nutr. 22 : 609-20. 1941.
10. Larmour, R. K., and J. W. G. MacEwen. The chemical composition of Russian thistle (*Salsola pestifer* A. Nels). Sci. Agr. 18 : 695-699. 1938.
11. Mitchell, R. L. The spectrophotometric analysis of soils, plants and related materials. Commonwealth Bureau of Soil Sci., Tech. Comm. No. 44. 1948.
12. Piper, C. S. Soil and plant analysis. Univ. Adelaide. 1942.
13. Recommended nutrient allowances for domestic animals. IV. Recommended nutrient allowances for beef cattle. Nat. Research Council, Washington, D.C. 1945.
14. Recommended nutrient allowances for domestic animals. III. Recommended nutrient allowances for dairy cattle. (*Revised*). Nat. Research Council, Washington, D.C. 1950.
15. Russell, F. C. Minerals in pasture. Deficiencies and excesses in relation to animal health. Imp. Bur. Animal Nutr., Tech. Comm. No. 15. 1944.
16. Theiler, A., H. H. Green, and P. J. DuToit. Minimum mineral requirements of cattle. J. Agr. Sci. 17 : 291-314. 1927.
17. Willard, H. H., and L. H. Greathouse. The colorimetric determination of manganese by oxidation with periodate. J. Amer. Chem. Soc. 39 : 2366-2377. 1917.

THE SULPHUR REQUIREMENTS OF MATURE RANGE EWES[1]

F. Whiting, S. B. Slen, L. M. Bezeau and R. D. Clark[2]

Experimental Station, Lethbridge, Alberta

[Received for publication October 2, 1953]

ABSTRACT

Six lots of 16 mature range ewes were fed, individually, pelleted rations in which the total sulphur content varied as follows: Lot 1, 0.08 per cent; Lot 2, 0.13 per cent; Lot 3, 0.17 per cent; Lot 4, 0.13 per cent; Lot 5, 0.17 per cent; and Lot 6, 0.13 per cent. Lot 1 was the basal ration and its sulphur content was that naturally occurring in the feedstuffs used. The additional sulphur was added to Lots 2 and 3 in the form of inorganic sulphates, to Lots 4 and 5 in the form of elemental sulphur and to Lot 6 in the form of methionine. There were no significant differences between the 6 lots over an 8-month period in the body gain of the ewes, in the quantity or quality of wool produced, in the weight or number of lambs produced, in the gain of the lambs to 6 weeks of age, in the total and inorganic sulphur content of the blood serum, in the total sulphur content of the colostrum, 7-day and 28-day milk, or in the sulphur content of the wool. The data indicate that the sulphur requirements of the mature ewe for wool and lamb production do not exceed 0.1 per cent of the total ration.

It has been established that the ruminant animal can utilize inorganic sulphates and elemental sulphur in the synthesis of sulphur-containing amino acids* (1, 2, 8, 12, 21, and 23). However, there is little information available on the sulphur requirements of this class of animal, or the relative effectiveness of the various forms of sulphur in meeting these requirements. Such information would be valuable if, due to the inclusion of urea or certain sulphur-low proteins in the ration, sulphur supplementation became necessary.

Thomas *et al.* (23) and Starks *et al.* (21) found that lambs fed low-sulphur purified rations (0.08 to 0.16 gm. sulphur daily) remained in negative sulphur and nitrogen balance and exhibited gradual failure of appetite, depraved appetite, emaciation, and finally death. When these rations were supplemented (approximately 1.8 gm. sulphur daily) with inorganic sulphates (23) or elemental sulphur (21) the lambs remained in positive sulphur and nitrogen balance and showed no symptoms of sulphur deficiency. The data of Hale and Garrigus (8) suggest that sulphate sulphur may be utilized better than elemental sulphur by sheep for the synthesis of cystine.

Steyn (22) reported that when sheep on pasture were given 5 gm. sulphur once, twice, or six times weekly there was an increase in wool production and body weight. Other workers obtained no response (4, 5, 16, 18, 19, and 24).

Garrigus *et al.* (7) reported a significant increase in wool production but no increase in body gain of feeder lambs when $\frac{1}{2}$ per cent elemental sulphur was added to a ration low in sulphur amino acids. When this ration was supplemented with $\frac{1}{2}$ per cent methionine there was a significant

[1]Contribution from the Division of Animal Husbandry, Experimental Farms Service, Canada Department of Agriculture, Ottawa.
[2]Animal Nutritionist, Wool Specialist, Assistant Animal Nutritionist, and Animal Husbandman, respectively.
*Migicovsky, B. B., S. B. Williams, S. B. Slen, and F. Whiting. Inorganic sulfur metabolism. *Unpublished data.* 1952.

increase in wool growth and body gain. Lofgreen *et al.* (11), on the other hand, found that the addition of 0.2 per cent sodium sulphate to a urea ration for feeder lambs was without effect on body weight, efficiency of feed utilization, nitrogen retention, serum sulphate levels, or wool growth. Madsen *et al.* (15) also concluded that the addition of sodium sulphate or methionine to a low-protein ration, supplemented or not supplemented with urea, had no significant effect upon wool production, rate of gain, or feed consumption. Loosli and Harris (13) reported that the addition of either methionine or sodium sulphate to a urea ration resulted in increased nitrogen retention but only the methionine increased the rate of growth.

Darlow *et al.* (3) and Du Toit *et al.* (4) failed to obtain a significant response in wool production or body growth by increasing the amount of cystine fed to wethers.

Jones *et al.* (9) found that supplementing a low-sulphur ration (0.1 per cent total sulphur) for dairy cows with sodium sulphate or methionine was without effect upon the efficiency of feed utilization.

This paper describes an experiment carried out during 1952-53 to assess the sulphur requirements of mature range ewes for lamb and wool production and to determine the effectiveness of methionine, inorganic sulphates, and elemental sulphur in meeting these requirements.

Table 1.—The physical composition of the mineral mixture

	Lots					
	1	2	3	4	5	6
Bonemeal (Kg.)	100	100	100	100	100	100
Salt (plain) (Kg.)	125	109	93	117.5	110	96
Limestone (Kg.)	25	15	5	25	25	25
Sodium sulphate (Kg.)	—	16	32	—	—	—
Calcium sulphate (Kg.)	—	8	16	—	—	—
Magnesium sulphate (Kg.)	—	2	4	—	—	—
DL-methionine (Kg.)[1,2]	—	—	—	—	—	29
Elemental sulphur (Kg.)	—	—	—	7.5	15	—
Manganese chloride (gm.)	100	100	100	100	100	100
Cuprous chloride (gm.)	70	70	70	70	70	70
Ferric citrate (gm.)	700	700	700	700	700	700
Cobalt chloride (gm.)	100	100	100	100	100	100
Potassium iodide (gm.)	30	30	30	30	30	30

[1] Part of the methionine used in this experiment was donated by E. I. du Pont de Nemours and Company, Wilmington, Delaware.

[2] The methionine was mixed with the minerals to facilitate its addition to the ration.

PROCEDURE

Ninety-six mature-grade Romnelet ewes were divided into 6 lots, uniform with respect to body weight, fleece weight, and type, on September 23, 1952. A basal ration which differed only in the composition of the mineral mixture was fed to all lots. It was composed of the following:

	Per cent
Wheat straw	50
Dried molasses beet pulp	10
Sugar (sucrose)	10
Starch	10
Barley	14
Urea	2.5
Corn oil	1.5
Mineral mixture	2.0

The composition of the mineral mixture is shown in Table 1.

The ration contained approximately 11 per cent total protein (N $\times$ 6.25) on an air dry basis. The entire ration was ground, mixed, and pelleted. All ewes were fed individually to appetite with the restriction that total feed consumption between lots was kept approximately equal. Six weeks before lambing was to begin the urea content of the ration was increased to 3 per cent to result in a ration containing approximately 12.5 per cent total protein. Many of the ewes reduced their feed consumption after this increase in urea and it was necessary to feed daily approximately 1.0 pound of oats (0.14 per cent total sulphur) and 0.4 pound of wheat straw (0.1 per cent total sulphur) in addition to the pellets in order to keep the ewes on feed. As all natural supplies of water contained appreciable quantities of sulphates, all water offered to the ewes during this experiment was distilled to remove sulphates. Wood shavings were used as bedding. The ewes were bred during November and December using three rams that were rotated from pen to pen every 12 hours. Lambing started on April 15. Each ewe was weighted at 28-day intervals and within 12 hours after lambing. The lambs were weighed at birth and on the 7th, 14th, 21st, 28th, 35th, and 42nd day after birth.

Blood samples were obtained from six ewes in each lot, by venipuncture, approximately 6 weeks before lambing and again 6 weeks after lambing. Colostrum samples were obtained from six ewes in each group before the lambs had nursed, and milk samples were obtained on the 7th and 28th day after lambing. The blood samples were analysed for total and inorganic sulphur, and the milk and colostrum samples for total sulphur, nitrogen, and fat content.

All samples, except the feed samples, were analysed for sulphur by the methods outlined by Snell and Snell (20). These methods employ nitric and perchloric acid for digestion, and turbidimetry for measuring the sulphur content. Feed samples were oxidized in a Parr oxygen bomb followed by turbidimetric measurement of the sulphur.

Each ewe had a 2 $\times$ 2 cm. area outlined by tattoo on the right shoulder. Weight of clean wool, fibre length, fibre thickness, and density of fibres on a given area were determined on the wool clipped from these areas at 60-day intervals. Total sulphur content was determined on the last

TABLE 2.—AVERAGE FEED CONSUMPTION, BODY WEIGHT, AND WOOL GROWTH AS INFLUENCED BY THE SULPHUR CONTENT OF THE RATION

	Lot 1 (basal)	Lot 2 (sulphate)	Lot 3 (sulphate)	Lot 4 (elemental)	Lot 5 (elemental)	Lot 6 (methionine)
Number of ewes that lambed and raised lambs to 6 weeks of age	14	13	14	12	15	13
Body weights of ewes—						
Initial weight (lb.)	116	118	116	114	116	115
Weight 6 weeks before lambing (lb.)	127	136	136	135	129	128
Weight after lambing (lb.)	130	134	134	136	132	133
Weight 8 weeks after lambing (lb.)	101	104	105	106	100	104
Daily feed consumption (lb.)—						
Until 6 weeks before lambing	2.7	2.8	2.7	2.8	2.7	2.7
During 6 weeks before lambing	2.9	3.2	3.2	3.2	3.2	3.2
During first 8 weeks' lactation	3.1	3.2	3.1	3.1	3.0	3.2
Sulphur content of ration (per cent)—						
Until 6 weeks before lambing	0.08	0.13	0.17	0.13	0.17	0.13
Last 6 weeks before lambing	0.10	0.13	0.15	0.13	0.15	0.13
During lactation	0.10	0.13	0.15	0.13	0.15	0.13
Wool production—						
Clean fleece weight (lb.)	3.5	3.5	3.6	3.8	3.5	3.6
Wool from a measured area (4 sq. cm.) (60 days' growth)—						
Weight of wool (mgm.)	82	93	92	96	89	92
Fibre thickness (u)	23	22	22	23	22	23
Fibre length (mm.)	20	19	20	20	10	19
Density of fibres (000's per sq. inch)	11.8	13.4	13.6	13.0	12.9	12.9

60-day sample of wool obtained. The ewes were shorn at the completion of the experiment and weights of scoured wool determined from the raw fleeces.

RESULTS AND DISCUSSION

The data on weights of ewes, feed consumption, wool production, sulphur content of the ration, wool, blood serum and milk, birth weights of the lambs, and their weights at 6 weeks of age, as influenced by the amount and form of sulphur in the ration, are shown in Tables 2, 3 and 4.

Although the ewes did not consume sufficient feed to supply the recommended allowances of total digestible nutrients for sheep of this weight (14) they made satisfactory gains in body weight between breeding and lambing. However, they lost considerable weight during lactation. Total wool production was below normal for these ewes. This may be due to the reduced feed consumption.

It is evident from Tables 2, 3 and 4 that amount or form of sulphur in the ration had no significant ($P < 0.05$) effect upon body weight gains, feed consumption, or wool production of the ewes, the sulphur content of the blood, milk, or wool, or the birth weights and weights at 6 weeks of age of the lambs.

The data in Table 3 on the sulphur content of the blood serum and wool are similar to those reported by other workers (11, 22, and 24). Data on the sulphur content of sheep colostrum and milk were not found in the literature available.

The results obtained in this study indicate that a ration containing between 0.08 and 0.1 per cent total sulphur (1.0 to 1.4 gm. daily) supplies sufficient sulphur to meet the needs of mature ewes for normal lamb and wool production. Since the basal ration supplied sufficient sulphur for these ewes, it was not possible from this experiment to determine the minimum requirements or the relative effectiveness of the different forms. The data of Thomas *et al.* (23) and Starks *et al.* (21) indicated that the sulphur requirements of feeder lambs was greater than 0.16 gm. daily (0.06 per cent of the ration) but not over 1.8 gm. daily (0.70 per cent of the ration). Jones *et al.* (9) reported that a ration for dairy cows, containing 0.1 per cent sulphur, was adequate for milk production. Tentatively, and until more data are available, it can be concluded that the sulphur requirement of the mature ewe and possibly all ruminant animals does not exceed 0.1 per cent of the total ration. Since practically all common feedstuffs contâin more than 0.1 per cent total sulphur (18) there seems to be little or no value in adding sulphur supplements to the rations of ruminant animals. However, rations compounded from feeds grown on sulphur-deficient soils may require sulphur supplements (26).

The data presented in this paper do not agree with those of Loosli and Harris (13), Lofgreen *et al.* (10), Garrigus *et al.* (7), Gallup *et al.* (6), and others who have reported beneficial results from adding methionine to a ration for sheep in which urea contributes a considerable portion of the total nitrogen.

TABLE 3.—SULPHUR CONTENT OF THE COLOSTRUM, MILK (FAT-FREE BASIS), BLOOD SERUM, AND WOOL AS INFLUENCED BY AMOUNT AND TYPE OF SULPHUR SUPPLEMENTATION

	Lot 1 (basal)	Lot 2 (sulphate)	Lot 3 (sulphate)	Lot 4 (elemental)	Lot 5 (elemental)	Lot 6 (methionine)
Colostrum and milk (mgm. per 100 ml.)—						
Colostrum	194 ± 38[1]	199 ± 38	208 ± 42	194 ± 60	212 ± 30	191 ± 53
7-day milk	42 ± 9	38 ± 5	39 ± 12	42 ± 6	31 ± 5	39 ± 8
28-day milk	41 ± 7	44 ± 5	35 ± 13	41 ± 11	42 ± 9	39 ± 9
Blood serum 6 weeks before lambing (mgm. per 100 ml.)—						
Total sulphur	94	88	92	97	90	91
Inorganic sulphur	4.0	4.7	4.4	5.0	5.0	5.0
Blood serum 6 weeks after lambing (mgm. per 100 ml.)—						
Total sulphur	80	92	89	86	87	90
Inorganic sulphur	3.5	5.8	5.1	6.5	4.5	5.5
Wool (mgm. per 100 gm.)—						
Total sulphur	3.3	3.0	3.0	3.4	3.4	3.4

[1]Standard deviation.

TABLE 4.—AVERAGE BIRTH WEIGHTS AND WEIGHTS AT 6 WEEKS OF AGE OF THE LAMBS AS INFLUENCED BY AMOUNT AND KIND OF SULPHUR IN THE RATION OF THE EWES

	Lot 1 (basal)	Lot 2 (sulphate)	Lot 3 (sulphate)	Lot 4 (elemental)	Lot 5 (elemental)	Lot 6 (methionine)
Birth weights (lb.)—						
Singles	12.4 (9)[1]	12.3 (10)	11.7 (11)	12.0 (12)	12.0 (11)	12.7 (10)
Twins	8.6 (12)	9.5 (10)	9.5 (10)	9.5 (6)	9.0 (10)	9.5 (10)
All lambs	10.2	10.9	10.7	11.2	10.6	11.1
Weights at 6 weeks (lb.)—						
Singles	29.0 (8)	31.2 (8)	31.0 (10)	31.4 (10)	28.9 (10)	32.1 (9)
Twins raised single	24.9 (2)	26.4 (2)	30.8 (1)	21.8 (1)	—	28.6 (2)
Twins	19.0 (8)	18.7 (6)	22.4 (6)	19.9 (2)	19.4 (10)	19.4 (4)
All lambs	24.1	26.1	28.0	28.8	24.1	28.3

[1] Figures in parentheses are number of lambs.

REFERENCES

1. Block, Richard J., and Jakob A. Stekol. Synthesis of sulfur amino acids from inorganic sulfate by ruminants. Proc. Soc. Expl. Biol. Med. 73 : 391-394. 1950.
2. Block, Richard J., Jakob A. Stekol, and John K. Loosli. Synthesis of sulfur amino acids from inorganic sulfate by ruminants. 2. Synthesis of cystine and methionine from sodium sulfate by the goat and by the microorganisms of the rumen of the ewe. Arch. Biochem. 33 : 353-363. 1951.
3. Darlow, A. E., V. G. Heller, and W. R. Felton. The effect of the ration on wool growth and on certain fleece characteristics. Okla. Agr. Expt. Sta. Bull. 220. 1934.
4. Du Toit, P. J., A. E. Malan, J. W. Groenewald, and M. L. Botha. The effect of different forms of sulfur in the diet upon the growth of wool production of sheep. Onderstepoort J. Vet. Sci. and Anim. Ind. 4 : 229-239. 1935.
5. Du Toit, P. J., A. E. Malan, J. W. Groenewald, and M. L. Botha. Studies in mineral metabolism. 34. The effect of sulphur upon the weight and wool production of sheep when food intake is not limited. Onderstepoort J. Vet. Sci. and Anim. Ind. 7 : 181-188. 1936.
6. Gallup, Willis D., L. S. Pope, and C. K. Whitehair. Value of added methionine in low-protein and urea rations for lambs. J. Anim. Sci. 11 : 572-577. 1952.
7. Garrigus, U. S., H. H. Mitchell, W. H. Hale, and J. S. Albin. The value of elemental sulfur in a methionine deficient sheep ration. J. Anim. Sci. 9 : 656-657. (*Abstract*). 1950.
8. Hale, W. H., and U. S. Garrigus. Synthesis of cystine in wool from elemental sulfur and sulfate sulfur. J. Anim. Sci. 12 : 492-496. 1953.
9. Jones, I. R., J. R. Haag, and P. H. Weswig. The relation of sulfur compounds to lactation in ruminants. J. Dairy Sci. 35 : 503. (*Abstract*). 1952.
10. Lofgreen, G. P., J. K. Loosli, and L. A. Maynard. The influence of protein sources upon nitrogen retention by sheep. J. Anim. Sci. 6 : 343-347. 1947.
11. Lofgreen, G. P., W. C. Weir, and J. F. Wilson. Gains in weight, nitrogen retention, and wool growth of lambs fed a ration containing urea supplemented with sodium sulfate. J. Anim. Sci. 12 : 347-352. 1953.
12. Loosli, J. K., H. H. Williams, W. E. Thomas, Fent H. Ferris, and L. A. Maynard. Synthesis of amino acids in the rumen. Science 110 : 144-145. 1949.
13. Loosli, J. K., and L. E. Harris. Methionine increases the value of urea for lambs. J. Anim. Sci. 4 : 435-437. 1945.
14. Pearson, P. B., H. M. Briggs, W. G. Kammlade, J. I. Miller, and R. F. Miller. Recommended nutrient allowances for sheep. National Research Council, Washington, D.C. 1949.
15. Madsen, Milton A., Lorin E. Harris, and Edward A. Nelson. Wool growth of weanling lambs fed various levels of urea, methionine, and sulfur. Proc. Annual Meeting, West. Section Amer. Soc. Anim. Prod. 4 : xxxii-1 to xxxii-8. 1953.
16. Marston, H. R. Studies on the relationship between nutrition and wool production of Merino sheep. J. Agr. Sci. 25 : 103-131. 1935.
17. Morrison, F. B. Feeds and feeding, pp. 1153-1159. 21st ed., Morrison Publishing Co., Ithaca, N.Y. 1948.
18. Peirce, A. W. The effect on the body weight and wool production of Merino sheep of adding sulfur to the diet. J. C.S.I.R. 6 : 294-298. 1933.
19. Seddon, H. R., and W. E. Chamberlain. On the effect of the administration of sulfur to sheep. Australian Vet. J. 9 : 154-159. 1933.
20. Snell, Foster Dee, and Cornelia T. Snell. Colorimetric methods of analysis, Vol. 2, 3rd ed., pp. 764-769. D. Van Nostrand Company, Inc., Toronto. 1949.
21. Starks, P. B., W. H. Hale, U. S. Garrigus, and R. M. Forbes. The utilization of feed nitrogen by lambs as affected by elemental sulfur. J. Anim. Sci. 12 : 480-491. 1953.
22. Steyn, D. G. The effect of sulfur on Merino sheep. 17th Rpt. Dir. Vet. Ser. and An. Ind., Pretoria, pp. 481-492. 1931.
23. Thomas, W. E., J. K. Loosli, H. H. Williams, and L. A. Maynard. The utilization of inorganic sulfates and urea nitrogen by lambs. J. Nutr. 43 : 515-523. 1951.
24. Underwood, E. J. The effect on body weight and wool growth of sulphur feeding to Merino sheep. J. Dept. Agr. W. Australia 11 : 507-511. 1934.
25. Weir, W. C., and V. V. Rendig. Studies on the nutritive value for lambs of alfalfa hay grown on a low sulfur soil. J. Anim. Sci. 11 : 780-781. (*Abstract*). 1952.

DEVELOPING CREEPING-ROOTED ALFALFA FOR PASTURE[1]

D. H. HEINRICHS[2]

Dominion Experimental Station, Swift Current, Sask.

[Received for publication August 28, 1953]

ABSTRACT

A breeding project is in progress at the Dominion Experimental Station, Swift Current, Saskatchewan, which aims to develop a creeping-rooted, drought-resistant alfalfa variety for the dry plains region of Canada. Progress has been made and strongly creeping-rooted lines have been developed. The breeding work has been conducted on the basis of combining ability for the creeping-rooted character. Results on this phase of the project are presented in the paper along with some correlation coefficients between important characters. It was concluded that breeding for the creeping-rooted character on the basis of combining ability is sound since the character itself appears to be of a quantitative nature.

INTRODUCTION

The importance and necessity of developing a hardy pasture legume for the Great Plains of the United States and Canada has been recognized by many workers. Keller (5), in an article entitled, "Wanted: A Paragon for the Range", states that no legume has yet been found that appears promising for rangelands over extensive areas and as a result reseeding practices usually include only grasses.

Of all the legumes tested in the prairie region of Western Canada, alfalfa has proved to be the most productive and persistent. However, even the hardiest common varieties, such as Ladak and Grimm, do not persist through a series of dry years, and if pastured all alfalfa plants disappear relatively quickly from the stand even in wet years. In order to maintain pastures and rangelands in a productive condition, it is important that a hardier variety than those now available be developed.

There is evidence to indicate that alfalfas which have underground crowns and creeping rootstocks are more drought-resistant and long-lived than bunch rooted types, especially under grazing. The yellow-flowered alfalfas (*Medicago falcata* L.) which Hansen (3, 4) introduced to the United States during the course of three trips to Siberia (1897-1898, 1906, and 1908-1909) appear to have these characteristics. In the Volga River region in the midst of a vast steppe which suffered severe droughts, experiments have shown that, although cultivated *Medicago sativa* L. winterkilled frequently and did not endure pasturing, *M. falcata* was perfectly hardy and endured pasturing for at least ten years. In Northern Siberia Hansen (3) found yellow-flowered alfalfa growing wild north of Yakutsk where winter temperatures of −85 degrees have been recorded.

Following their introduction, the yellow-flowered alfalfas were investigated further. Oakley and Garver (8) give an exhaustive description of *M. falcata* and discuss its relationship with *M. sativa*. They comment that the greatest agronomic possibilities of the species appear to be in the field of selection and hybridation with forms of *M. sativa*. Oakley (7), Garver (2), and Southworth (9) studied various root systems of alfalfa. The

[1] Contribution from Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada.
[2] Agricultural Research Officer, Forage Plants Section, Dominion Experimental Station, Swift Current, Sask.

general conclusions arrived at by these workers are pretty well summarized by Southworth (9). He states, "The hardiness of alfalfa depends very largely on its root system. Plants possessing a branched root system are much better able to withstand heaving than those having only a single tap-root, no matter how great its length may be. Those plants which have the power to produce rooting underground stems are able to renovate themselves, and after the death of the main rootstock are capable of keeping up a separate existence quite independent of the parent rootstock. When alfalfa has the habit of spreading by means of root proliferation, we have a form of spreading and multiplying in a vegetative manner which promises to give to the plant greater powers of resistance to cold and also greater powers of recuperation from injury than is possessed by even true rhizomes, and we venture to hope that these properties will render it possible to grow good crops in adverse climatic conditions under which it would be quite impossible to raise common alfalfa".

Various strains of yellow-flowered alfalfas were distributed to farmers and experimental institutions in the United States and Canada shortly after 1910. According to Hansen (3) the strongest of these strains was the one from Semipalatinsk. However, none of the strains became commercially important, probably because the seed set was very poor and the little seed that did set shattered readily. At the present time only a few fields of yellow-flowered alfalfa are known in North America. The writer is familiar with two fields in Saskatchewan, both of which originated from small amounts of seed obtained from Hansen. At one location, alfalfa planted in 1916 has persisted in a cow pasture to this day.

The possibilities of using the yellow-flowered Siberian alfalfa strains for developing hardier alfalfa varieties for the Canadian prairie region were recognized by a number of forage crop breeders in Canada, notably S. E. Clarke, retired, and J. L. Bolton, Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan. As a result breeding work was undertaken at the Dominion Experimental Station, Swift Current, Saskatchewan, in 1938 which had as its objective to combine the hardy qualities of *M. falcata* with the good seed-producing habits and the growth vigour of common varieties.

This paper reviews the breeding history and presents some of the results and achievements. Good progress has been made and certain creeping-rooted lines appear to be very promising for dryland ranges and pasture seedings.

MATERIAL AND METHODS

Early Phase

In 1934 Ladak, Grimm, and Siberian (*M. falcata*) were seeded at the Dominion Experimental Station, Swift Current, Saskatchewan, in a 4-replicate test on dry land. The seed of Siberian was obtained from N. E. Hansen, South Dakota State College, Brookings. The strain identity is unknown. The Ladak seed came from a foundation seed plot at the Range Experiment Station, Manyberries, Alberta. During the years 1936, 1937, and 1938, heavy winter-killing occurred in certain plots, presumably due mainly to drought, and in 1938 the relative stand of the varieties was as follows: Grimm, 5 per cent; Ladak, 25 per cent; and Siberian, 100 per cent.

During 1938, selections were made from the mass population of the surviving Ladak and Siberian plants in the above test, and in 1939 a spaced-plant, dryland nursery consisting of 2,200 Ladak and 500 Siberian plants was established. From this nursery a more exacting selection was made, the criteria being creeping-rooted habit and upright stem growth in the Siberian plants, and vigour of growth and high seed yield in the Ladak plants. In subsequent years these selections were evaluated on the basis of selfed progenies, controlled crosses, and back crosses. This program provided new populations for further selection.

In the spring of 1940 clones and open-pollinated seed were obtained from G. G. Moe, University of British Columbia, Vancouver, British Columbia. The material was some of the same that went into the making of the rhizomatous variety Rhizoma (6). Of 2,300 plants established, only 45 survived the unusually severe winter of 1940-41. Several of these 45 plants were used in crosses with Siberian and the resultant progenies provided some useful material for further selections.

In 1947, when the alfalfa breeding program was intensified a population of approximately 10,000 plants was available for selection. Many of the progeny lines contained a large proportion of strongly creeping-rooted plants and the best of these formed the nucleus for the recent phase of the breeding program which has been based chiefly on the study of the combining ability for the creeping-rooted character and satisfactory yield.

FIGURE 1. A creeping-rooted plant two years after it was transplanted to the field. The protractor-like instrument provided a convenient and rapid method for measuring the spread of the plants. It is calibrated near the top for convenient reading.

Recent Phase (1947-51)

The plants selected in 1947 were all creeping-rooted in varying degrees. The pedigree of the selected plants traced back on the *M. falcata* side to one or other of eight original selections. Only two of these selections were truly creeping-rooted, the other six being merely broad-crowned. On the *M. sativa* side the selections traced back to one or other of 17 Ladak and three Rhizoma plants. Since many of the 1948 selections excelled the *M. falcata* parents in the creeping-rooted habit, it would appear that certain *M. sativa* plants possess hidden factors for creeping-rootedness which, by complementing the main factors from the *M. falcata* plants, produced the improved expression of the character in many progenies. The continuous range of variation in the progenies suggests that creeping-rootedness is a quantitatively inherited character.

On the basis of these results breeding work was carried out to determine the combining ability of individual plants. The method followed was to cross a particular plant on six to ten unrelated plants and test the progenies. The general combining ability of a plant was calculated from the average performance of all cross-pollinated progenies, while an indication of its specific combining ability was obtained from the performance of each cross-progeny line.

In 1947 the extent of spread of each plant was measured with a protractor-like instrument (Figure 1), while in later years it was rated from one to five, one representing the greatest spread and five a bunch crown. All the pod and seed production characters, vigour, and forage value were rated from one to five, the lower number representing the more desirable class.

All progeny tests were replicated at least twice and the plants were started in the greenhouse and space planted in the field.

The term "family" when used refers to all progenies from one plant.

EXPERIMENTAL RESULTS

Description of Creeping-rooted Plants

All plants selected in 1947, and later through the course of the breeding program, were truly creeping-rooted and not merely rhizomatic. Histological studies presently being made show that the horizontal rootstocks from which green shoots arise are roots and not rhizomes. The creeping rootstocks are generally found 4 to 8 inches below the surface of the ground (Figure 2). They send up green shoots at intervals, each of which is capable of becoming an independent plant. Some plants are dense creepers, others lax; that is, certain plants produce more green shoots per unit area than others. Also, in some plants the spread is extensive while in others it remains restricted. A detailed analysis of the excavated plant No. 2, partially shown in Figure 2, established that 286 separate surface crowns had arisen within a period of three years in this plant. The spread of three-year-old plants often exceeds 9 feet (Figure 3).

Combining Ability

Some results depicting the combining ability for the creeping-rooted character are presented in Table 1. These data are typical and show that

Plant 1. Plant 2. Plant 3.

FIGURE 2. Excavated plants; each excavation is 2 feet wide and 4 feet deep; the length varies with the plant and is indicated on the mount.

Plant 1—Ladak.

Plant 2—(Ladak × Siberian) × (Ladak × Siberian) Siberian.

Plant 3—Ladak × (Ladak × Siberian).

NOTE: The creeping rootstocks are considerably deeper in Plant 3 than in Plant 2. The latter, however, has a denser creep.

FIGURE 3. A strongly creeping-rooted plant which has spread 9 feet in three years. The pedigree of this plant is (Ladak × Siberian) × (Ladak × Siberian) Ladak. The flower colour of this plant is blue.

TABLE 1.—PERCENTAGE OF CREEPING-ROOTED PROGENIES OBTAINED FROM INTERCROSSES OF STRONGLY CREEPING-ROOTED PLANTS; THE COMBINING ABILITY DATA FOR SIX TYPICAL PLANTS ARE PRESENTED OUT OF A TOTAL OF 58 STUDIED IN A TEST PLANTED IN MAY 1948; 26 PROGENIES PER CROSS WERE STUDIED IN TWO REPLICATES

Cross No.	Parent plant		Progenies creeping-rooted		
	Female	Male	1949 per cent	1950 per cent	Increase per cent
70	24716 (L × S) (L × S)L	24730	50	87	37
73	24716	24742	53	57	4
75	24716	24747	48	86	38
76	24716	24748	75	95	20
80	24716	24764	59	82	23
84	24716	24779	14	71	57
	Average		49	79	29
150	24734 (L × S) (L × S)L	24742	21	89	68
152	24734	24747	39	78	39
153	24734	24748	30	68	38
154	24734	24750	50	68	18
157	24734	24759	5	47	42
158	24734	24764	60	95	35
	Average		34	74	40
437	24736 (L × S) (L × S)L	24744	60	65	5
440	24736	24753	53	66	13
443	24736	24757	45	45	0
444	24736	24760	57	67	10
445	24736	24762	40	50	10
446	24736	24767	100	100	0
	Average		59	65	6
188	24742 (L × S) (L × S)L	24747	32	56	24
189	24742	24751	46	87	41
192	24742	24774	35	54	19
194	24742	24779	60	78	18
150	24742	24734	21	89	68
266	24742	24753	50	96	46
	Average		40	76	36
204	24747 (R × L) (L × S)S	24748	14	60	46
205	24747	24750	62	66	4
106	24747	24721	0	33	33
122	24747	24728	80	100	20
138	24747	24731	73	100	27
268	24747	24753	73	95	22
	Average		50	75	25
232	24754 (L × S)F_2	24746	61	87	26
233	24754	24774	60	80	20
234	24754	24777	50	96	46
235	24754	24778	58	96	38
236	24754	24779	50	92	42
168	24754	24737	57	95	38
	Average		56	91	35

NOTE: L = Ladak; S = Siberian (*M. falcata*); R = Rhizoma.

any given plant when crossed with a number of unrelated creepers gives a series of progenies differing widely in their percentages of creeping-rooted plants. The data also show that the average performance of the various plants is markedly different. As seen in Table 1, plant 24754 had the best general combining ability with an average of 91 per cent of its progenies rated as creeping type in the second year. The specific combining ability of this plant in the six combinations was uniformly good. Plant 24736 which showed the lowest general combining ability also showed the greatest variability in its specific combinations.

The data presented in Table 1 further indicate that the creeping-rooted character does not express itself in some plants until they are two years old. In this regard the progenies from the six plants differ considerably from each other. For example, the progenies from plant 24736 showed an average increase of creeping plants of only 6 per cent from 1949 to 1950 as compared with a 40 per cent increase for the progenies from plant 24734.

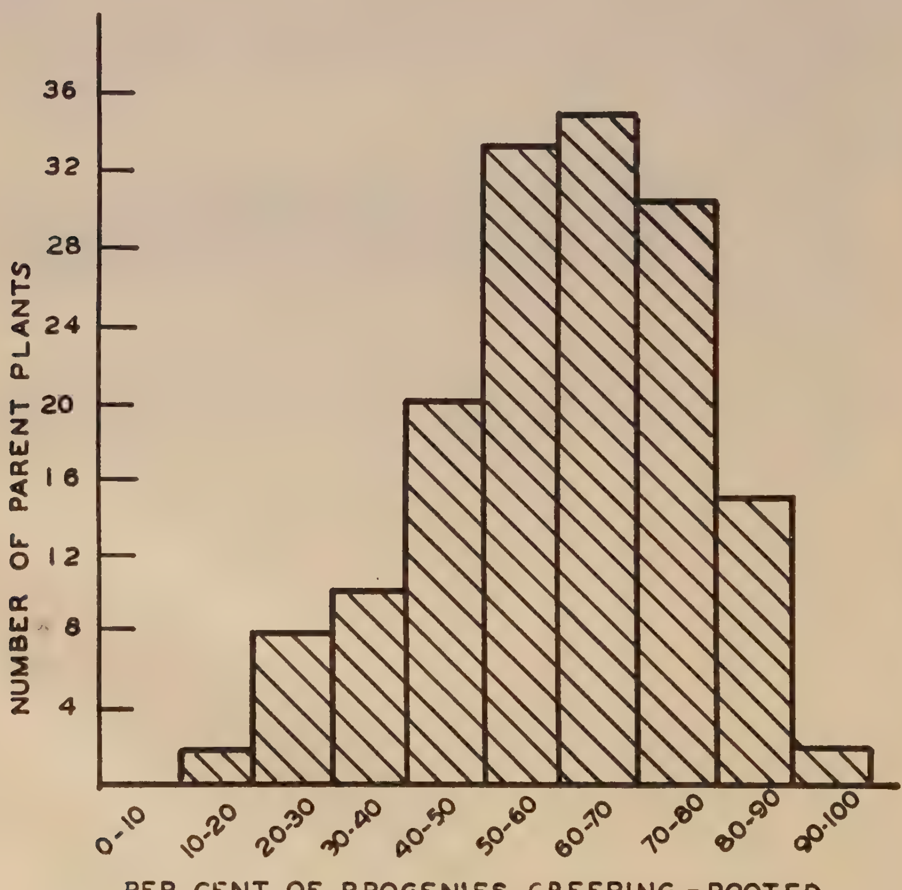


FIGURE 4. Frequency distribution of 141 creeping-rooted plants according to the per cent of creeping-rooted plants their progenies contained.

TABLE 2.—SOME IMPORTANT CORRELATION COEFFICIENTS INVOLVING THE CREEPING-ROOTED CHARACTER

Comparison	N	r
Spread of selected plant in inches and percentage of cross-pollinated progenies creeping-rooted	55	+ 0.59**
Percentage of cross-pollinated progenies creeping-rooted when one year old and two years old	54	+ 0.78**
Percentage of cross-pollinated progenies creeping-rooted and percentage of inbred progenies creeping-rooted when two years old	22	+ 0.69**

** Significant at the 1 per cent level.
N = number of families studied.

The histogram (Figure 4) shows the frequency distribution of 141 creeping-rooted plants classified according to the percentage of creeping-rooted plants their cross-pollinated progenies contained. On the average, approximately 200 cross-pollinated progenies from each of the 141 plants were studied. These progenies came from seed obtained from about ten crosses of creeping-rooted plants. In each cross a particular plant was used as the common parent, i.e., as exemplified in Table 1. The histogram shows that the distribution is that expected for a quantitative character. However, it should be noted that about three-fourths of the parent plants produced more than 50 per cent creeping-rooted plants within their progenies.

The correlation coefficients presented in Table 2 show that (1) a higher percentage of creeping-rooted plants can be expected in the progenies from plants with a wide horizontal spread than from those with a restricted spread; (2) the relationship between the percentage of plants creeping-rooted when one year old and when two years old was sufficiently close to make results on creeping-rootedness quite reliable from the one-year old plants; and (3) both cross-pollinated and self-pollinated progenies are satisfactory for evaluating the parent plants for transmissibility of the creeping-rooted character.

In Table 3 the performance of F_1 progenies is illustrated. Every one of the Siberian plants produced a number of strongly creeping-rooted progenies, some having a spread of 8 feet in the third year. However, several of them transmitted this character to more of their progenies than did others. The data also indicate that creeping-rootedness was not expressed by many progenies until they were two years old.

The seed yield results given in the last column of Table 3 show that very great variability exists in specific combining ability for seed yield. However, in general, the plants with poor specific combining ability also were poor in general combining ability for seed yield. For example, the Ladak plant 2462 produced very poor seed yielding progenies in all crosses.

Association of Plant Characters

Simple correlation coefficients, calculated from individual plant data in eight families, are presented in Table 4. The families selected varied

TABLE 3.—PERCENTAGE OF CREEPING-ROOTED PROGENIES FROM SIX BROAD-CROWNED SIBERIAN PLANTS CROSSED ON SEVERAL LADAK PLANTS PLANTED IN 1948 IN TWO REPLICATES; EACH PLOT CONTAINED FROM 10 TO 13 PLANTS

Cross No.	Parent plant		Progenies creeping-rooted			Seed yield, pound/acre of progeny line
	Male plant Siberian	Female plant Ladak	1949 per cent	1950 per cent	Difference 1949–1950 per cent	
22	24770	2461	5	5	0	64
23	24770	2462	13	13	0	27
24	24770	2463	0	7	7	37
25	24770	2464	0	0	0	13
26	24770	2465	0	0	0	69
		Average	4	6	3	42
28	24771	2461	29	29	0	64
30	24771	2463	0	29	29	59
31	24771	2464	0	33	33	11
32	24771	2465	6	25	19	85
		Average	9	29	20	55
40	24773	2461	17	33	16	117
41	24773	2462	0	55	55	11
42	24773	2463	0	29	29	75
43	24773	2464	0	6	6	16
44	24733	2465	0	0	0	64
		Average	3	25	21	56
5	2466	2461	0	0	0	85
7	2466	2462	0	0	0	6
8	2466	2463	13	13	0	64
6	2466	2464	6	6	0	21
4	2466	2465	39	39	0	32
		Average	11	11	0	42
17	2479	2462	15	23	8	11
18	2479	2463	5	26	21	59
19	2479	2464	10	10	0	7
		Average	10	20	10	26
34	24772	2461	5	11	6	37
36	24772	2463	44	44	0	37
38	24772	2465	0	13	13	27
		Average	16	22	6	34

over a wide range for each character. The creeping-rooted character, and characters having a direct bearing on seed yield, were considered.

The data show that in most families there was no significant correlation between the degree of creeping-rootedness and seed yield characters. The degree of pod coiling and seed set was significantly positively correlated in all families although the association was relatively unimportant in all but two families. The degree of pod coiling and shatterability was significantly negatively correlated in all but one family, and in at least three families

TABLE 4.—CORRELATION COEFFICIENTS BETWEEN VARIOUS CHARACTERS IN EIGHT FAMILIES OF ALFALFA

Comparison	Cross-pollinated progenies from plant							
	24714 R × (L × S)	24716 (L × S) (L × S)L	24720 (L × S) (L × S)L	24751 (L × S)F_2	24760 L(L × S)	24763 L(L × S)	24764 S(L × S)	24776 S(L × S)
Creeping rootedness and degree of pod coiling	− 0.023	+ 0.138	− 0.096	− 0.144	− 0.011	+ 0.063	+ 0.125	+ 0.234
Creeping rootedness and seed set	+ 0.003	− 0.150	− 0.728**	+ 0.243*	− 0.002	+ 0.128	− 0.019	+ 0.280*
Creeping rootedness and shatterability	+ 0.188	− 0.588**	+ 0.196	+ 0.072	+ 0.409**	+ 0.039	+ 0.166	− 0.263*
Degree of pod coiling and seed set	+ 0.299*	+ 0.364**	+ 0.303*	+ 0.395**	+ 0.539**	+ 0.365**	+ 0.574**	+ 0.432**
Degree of pod coiling and shatterability	− 0.743**	− 0.551**	− 0.384**	− 0.427**	− 0.718**	− 0.490**	− 0.403**	− 0.029
Seed set and shatterability	− 0.421**	− 0.651**	− 0.478**	− 0.194	− 0.499**	− 0.338**	− 0.472**	− 0.131
N	65	65	47	65	65	52	52	52

NOTE: R = Rhizoma; L = Ladak; S = Siberian (*M. falcata*).
N = number of plants studied per family.
* Significant at the 5 per cent level; ** significant at the 1 per cent level.

that association was quite important. Seed set and shatterability were negatively correlated in all families but in two this correlation was not significant.

Winter Hardiness

Two 4-replicate tests planted in 1948, each of which included 45 lines from specific crosses, and the four varieties, Ladak, Grimm, Rhizoma, and Ferax, were harvested in September of that year. They were cut at this late date to increase their susceptibility to winter injury and this was augmented by exceptionally dry conditions both in the fall of 1948 and the spring of 1949. Stand notes were taken on June 13 and 14, about four weeks after a spring rain. At this time dead plants were easily recognized. Since stand notes had been taken in the fall it was possible to determine accurately the per cent winterkilling on each plot. The analysis of variance showed that there were highly significant differences in winterkilling between progeny lines. The four check varieties killed out 100 per cent in both tests, while only two of the lines killed out to this extent. Approximately 50 per cent of the lines showed less than 50 per cent winterkilling.

The correlation coefficient between winterkilling and degree of creeping-rootedness was found to be -0.60, significant at the 1 per cent level. In creeping-rooted plants, when the main crown was dead, numerous shoots appeared from creeping rootstocks at a considerable distance from the parent crown. It appeared that killing of the main crown actually stimulated development of green shoots from creeping rootstocks.

In a 2-acre breeding nursery of cross-pollinated progenies planted in 1948 considerable winter injury occurred after the second season. The severity of the damage was reflected in the vigour of the plants about three weeks after growth started in the spring. The correlation coefficient between the percentage of creeping-rooted progenies and vigour was $+0.51$, significant at the 1 per cent level. This indicated that the creeping-rooted plants were injured less than the non-creepers. In the same nursery practically no non-creeping-rooted plants could be found in 1952. The creepers had spread into all bare spots and there was a solid stand of alfalfa over the entire area.

DISCUSSION

In recent years much interest has centred around the development of spreading alfalfas for pasture. Several varieties of a rhizomatous nature, such as Rhizoma and Nomad, have been released for commercial use. These types appear to spread well under certain climatic conditions, but in an arid climate, such as exists in the prairie region of Canada, they spread very little probably because of inadequate surface soil moisture for much rhizomatic spreading to occur. The creeping-rooted alfalfa being developed at Swift Current, Saskatchewan, has its horizontal rootstocks from 6 to 8 inches under ground and the plants spread readily without moist surface conditions. These types have been found to be extremely hardy and should be particularly useful in arid regions.

In many plants the creeping-rooted character does not express itself fully until the plants are two years old and in order to detect all creepers

in a breeding nursery it would be necessary to grow the plants to an age of two years. This may be desirable, particularly in the initial stages of breeding when creepers are to be selected out of F_1 progenies. However, the fact that the correlation coefficient was + 0.78 between percentage of cross-pollinated progenies creeping-rooted when one year old and two years old from intercrosses of creeping-rooted plants, indicates that in the more advanced stages of breeding selections can be made within one-year-old populations.

Genetically, the creeping-rooted character appears to be quantitative with complementary factors playing a part, but the possibility of autotetraploid-type qualitative inheritance has not been ruled out. To date no combinations have been found to produce only creeping-rooted progenies, although in many cases the percentage is quite high, 90 or greater. It might be argued that creeping-rootedness would be diluted in advanced generations because of the non-creeping plants producing the greatest seed yield. Data have been obtained which indicate that this is not the case, that is, within progeny lines there is no correlation between high seed yield and non-creeping habit. Actually, the reverse might be the case because there is strong evidence to indicate that the creeping-rooted plants will eliminate the non-creepers from the stand by their superior hardiness and competitive ability. Fortunately, the creeping-rooted character does not appear to be associated with agronomically undesirable characters and for that reason it should be possible to develop creeping-rooted strains for various climatic regions. By a backcross method of breeding and periodic intercrossing of creeping-rooted selections, it should be possible to fix the character on *M. sativa* types.

Determination of combining ability of individual plants appears to be a good method for evaluating the capacity to transmit creeping-rootedness. These results are quite similar to those for other characters, Bolton (1) and Tysdal (10). The results from the breeding work in general indicate that the polycross method of breeding as outlined by Tysdal (10) should be satisfactory for evaluating selected creeping-rooted plants for breeding behaviour of the creeping-rooted character.

Synthetic lines developed at Swift Current are now under test at Stations across Canada. Their development has been based on results from combining ability tests. Primarily, they are creeping-rooted and hardy, suitable for dryland pastures. Their recovery is generally poorer than that of Ladak. In the majority the yellow flower colour predominates. It is hoped that one of these synthetic strains may be the "Paragon for the Range" requested by Keller (5).

ACKNOWLEDGMENTS

The writer wishes to acknowledge the contribution made to the project by former members of the staff—S. E. Clarke (retired), Vancouver, British Columbia; and J. L. Bolton Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan. Thanks are also due to Beatrice E. Murray, who has taken many of the field notes since 1947.

REFERENCES

1. Bolton, J. L. A study of combining ability of alfalfa in relation to certain methods of selection. Sci. Agr. 28 : 97-126. 1948.
2. Garver, S. Alfalfa root studies. U.S.D.A. Bull. 1087. 1922.
3. Hansen, N. E. Plant introductions. Bull. 224. Agr. Expt. Sta., South Dakota State College, Brookings. 1927.
4. Hansen, N. E. The wild alfalfas and clovers of Siberia with a prospective view of the alfalfas of the world. U.S.D.A. Bull. 150. 1909.
5. Keller, W. Wanted: A paragon for the range. U.S.D.A. Yearbook, pp. 347-351. 1948.
6. Nilan, R. A. Rhizoma alfalfa: Chromosome studies of the parent stocks. Sci. Agr. 31 : 123-126. 1951.
7. Oakley, R. A. Two types of proliferation in alfalfa. U.S.D.A. Circ. 115. 1913.
8. Oakley, R. A., and S. Garver. *Medicago falcata*—A yellow-flowered alfalfa. U.S.D.A. Bull. 428 : 1-67. 1917.
9. Southworth, W. A study of the influence of the root system in promoting hardiness in alfalfa. Sci. Agr. 1 : 5-9. 1921.
10. Tysdal, H. M., T. A. Kiesselbach, and H. L. Westover. Alfalfa breeding. Agr. Expt. Sta., Univ. of Nebraska, Res. Bull. 124. 1942.

THE RELATION OF AMMONIUM AND SULPHATE IONS TO MAGNESIUM DEFICIENCY IN TOBACCO[1]

E. T. McEvoy[2]

Central Experimental Farm, Ottawa, Canada

[Received for publication October 29, 1953]

ABSTRACT

An experiment was conducted with flue-cured tobacco in sand culture under conditions of controlled nutrient supply to study the relation of ammonium and sulphate ions in the nutrient medium to magnesium deficiency in tobacco. A series of 9 nutrient solutions was used, comprising 3 concentrations of ammonium nitrogen in factorial combination with 3 concentrations of sulphate sulphur. The total concentration of nitrogen was held at a constant level in all treatments by the necessary addition of nitrate.

The yield decreased with increased ammonium supply. Neither sulphate nor the interaction between sulphate and ammonium had a significant effect on yield. Plants which received high ammonium supply combined with either intermediate or high sulphate supply manifested chlorosis characteristic of magnesium deficiency. The content of magnesium in the leaves was depressed by each of NH_4^+ and $SO_4^=$ supply independently but to a greater degree by the former, and reached the lowest level at high NH_4^+ and high $SO_4^=$ supply combined.

INTRODUCTION

The disorder of tobacco commonly called sand drown has been shown to be caused by a deficiency of magnesium (2). It is called "sand drown", not because of actual drowning, but because it is most severe on deep, sandy soils and is accentuated by heavy rainfall. Since magnesium is a constituent of the chlorophyll molecule, a deficiency of this element interferes with the normal production of the green pigment and the leaves then assume a characteristic colour pattern. The green colour fades first from the tips and margins of the lowermost leaves. The tissue between the veins is affected most severely, while the veins and adjacent tissue tend to remain green. When severe, the disorder may advance over the entire leaf and progress upwards to the younger leaves. The diseased leaves, when cured, have a dull appearance and are thin, brittle and non-elastic with consequent impairment of the quality of the crop.

It was reported by W. W. Garner *et al.* (2) that sand drown of tobacco was aggravated by an increased sulphur supply from either sulphate of potash or sulphate of ammonia. Anderson *et al.* (1) reported that sand drown was severe on cigar tobacco plants grown on plots to which nitrogen was applied as sulphate of ammonia. In explanation, the latter workers suggested the possibility of the magnesium combining with the sulphate radical, forming magnesium sulphate, a highly soluble salt, which later was leached from the surface soil by heavy rains. In a previous paper (5), it was reported that symptoms of magnesium hunger, or sand drown, in tobacco grown in sand culture were associated with a high supply of ammonium nitrogen under conditions which precluded the possibility of a deficiency of magnesium being created by leaching. The nutrient solution

[1] Contribution from the Tobacco Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada.

[2] Principal Nutritionist, Tobacco Division.

containing magnesium was supplied continuously by the constant flow, drip method. Here was evidence that high ammonium supply aggravates magnesium hunger in tobacco.

Further investigation of the relation of ammonium and sulphate supply to magnesium hunger in tobacco was considered to be expedient. The present paper deals with the study of certain phases of the influence of ammonium and sulphate ions in the nutrient supply on the growth and composition of flue-cured tobacco grown in sand culture with particular reference to the relative influence of these two ions on the occurrence of magnesium deficiency in the plant.

MATERIALS AND PROCEDURE

Tobacco plants of the flue-cured variety White Mammoth were used in this experiment. The seedlings were grown in ground Nepean sandstone in 3-inch pots for 9 weeks. During this time, they were all supplied with a complete nutrient solution, previously developed (4), in which all the nutrient elements were at an optimum concentration for the best growth of tobacco. This solution was compounded from reagent grade salts of calcium nitrate, potassium dihydrogen phosphate, and magnesium sulphate. It contained Ca at a concentration of 240 p.p.m.; Mg, 45 p.p.m.; K, 195 p.p.m.; N, 168 p.p.m.; S, 60 p.p.m.; and P, 155 p.p.m. In addition, the minor elements B, Mn, and Zn were added to the solution at the rate of 0.5 p.p.m. and Fe was added at the rate of 3 p.p.m.

After 9 weeks' growth, the seedlings were transplanted to 3-gallon glazed, self-draining jars, each containing 40 pounds of ground Nepean sandstone. One plant was grown in each jar and received one litre of nutrient solution per day applied by the constant flow, drip method.

A series of 9 nutrient solutions was devised, comprising 3 concentrations of ammonium nitrogen (0, 88, and 175 p.p.m. of the nutrient solution) in factorial combination with 3 concentrations of sulphate sulphur (0, 118, and 236 p.p.m. of the nutrient solution). These nutrient solutions were made up from the reagent-grade chemicals KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4Cl, $Ca(NO_3)_2$, KNO_3, $NaNO_4$, $Mg(H_2PO_4)_2$, $MgSO_4$, $CaSO_4$, NaH_2PO_4, $CaCl_2$, and Na_2SO_4 to give the concentrations of nutrients shown in Table 1. In this series of solutions, the form, but not the concentration, of N was varied; as the NH_4^+-N was increased, the NO_3^--N was decreased correspondingly. Thus, the concentration of N was held constant at 175 p.p.m. of the nutrient solution in all 9 treatments. The concentration of each of N, K, Ca, and Mg was held constant at a level which previous studies (4) had shown to be within the optimum range for best growth of flue-cured tobacco. The concentrations of P varied slightly. The presence of Na and Cl in the nutrient solutions resulted from the necessity to use salts containing these elements in compounding the solutions. The minor element boron, manganese, and zinc were added to all solutions at the rate of 0.5 p.p.m. and iron was added at the rate of 3.0 p.p.m. Each treatment was replicated six times.

The plants were harvested two weeks after topping, and the green leaves were weighed. The leaves were diced and thoroughly mixed. Aliquots were weighed for chemical analyses and dried in an oven at 70° C.

TABLE 1.—COMPOSITION OF NUTRIENT SOLUTIONS

Treatment No.	Treatment		Concentration of elements, p.p.m.							
	NH_4-N	SO_4-S								
	p.p.m.	p.p.m.	N	P	K	Ca	Mg	S	Na	Cl
1	0	0	175	200	195	167	34	0	159	37
2	0	118	175	200	195	167	34	118	209	98
3	0	236	175	200	195	167	34	236	304	0
4	88	0	175	222	195	167	34	0	39	175
5	88	118	175	200	195	167	34	118	99	250
6	88	236	175	200	195	167	34	236	108	100
7	175	0	175	341	195	167	34	0	0	358
8	175	118	175	200	195	167	34	118	40	195
9	175	236	175	200	195	167	34	236	0	205

The methods used in the chemical analyses were the official methods of the A.O.A.C. (3) with one exception—total nitrogen was determined by the method of Pucher *et al.* (7).

RESULTS AND DISCUSSION

Growth of Plants

The plants grew better on the zero and intermediate NH_4^+ nitrogen than on the high NH_4^+ nitrogen. Symptoms of magnesium deficiency, chlorosis of the interveinal tissue on the lower leaves, were evident on the plants grown on 175 p.p.m. NH_4^+ nitrogen in combination with each of 118 and 236 p.p.m. $SO_4^=$ sulphur—treatments 8 and 9, respectively. The most severe symptoms were associated with combined high NH_4^+ and high $SO_4^=$ in treatment 9. In plants which received no sulphate, characteristic symptoms were obtained for sulphur deficiency—a pale green colour of the veins of the younger leaves.

Since Na and Cl were variable factors in the nutrient supply, the possibility that these elements had an influence on growth should be considered. In a previous experiment with flue-cured tobacco (6), the concentration of Na in the nutrient solutions ranged from zero to 116 p.p.m. and that of Cl from zero to 212 p.p.m. without inducing symptoms of magnesium deficiency or other observable effects. Also, unpublished data from another experiment conducted in the Tobacco Division show that the variation in the Na supply from zero to 200 p.p.m. was not a source of significant variation in the yield of flue-cured tobacco grown in sand culture. In addition, the supply of P in the present experiment was greater in treatment 7 than in the other treatments. In previous experiments (4), the concentration of P in the nutrient solutions ranged from 31 to 248 p.p.m. without inducing symptoms of magnesium deficiency. It

TABLE 2.—FRESH-WEIGHT YIELD PER PLANT IN GRAMS FROM THE VARIOUS TREATMENTS

Treatment No.	Treatment		Mean fresh weight in grams		
	NH_4-N p.p.m.	SO_4-S p.p.m.	Bottom leaves	Top leaves	Total leaves
1	0	0	322	445	767
2	0	118	307	398	705
3	0	236	360	423	783
4	88	0	346	364	710
5	88	118	368	347	715
6	88	236	282	337	619
7	175	0	215	173	388
8	175	118	192	155	347
9	175	236	232	148	380
L.S.D. (P = 0.05)					143

TABLE 3.—MEAN FRESH-WEIGHT YIELDS IN GRAMS FOR THE 9 TREATMENT-COMBINATIONS AND MEAN YIELD AT EACH FACTOR LEVEL, SHOWING MAIN EFFECTS OF NH_4-N AND SO_4-S

	0 p.p.m. SO_4-S	118 p.p.m. SO_4-S	236 p.p.m. SO_4-S	Means for main effect of NH_4-N
0 p.p.m. NH_4-N	765	706	783	751.3
88 p.p.m. NH_4-N	694	722	673	696.3
175 p.p.m. NH_4-N	389	348	380	372.3
Means for main effect of SO_4-S	616.0	592.0	612.0	—

L.S.D. (P = 0.05) between means for main effects = 56 grams.

is indicated, therefore, that the variation in the supply of these 3 elements did not have a significant influence on the development of magnesium deficiency symptons in the plants or otherwise seriously interfere with the interpretation of the results.

The fresh-weight data of the leaves are presented in terms of grams per plant in Table 2. An analysis of variance was performed on the fresh-weight data. The NH_4^+ nitrogen was a source of highly significant variation; the yield varied inversely with the NH_4^+ supply. The differences in yield associated with differences in the $SO_4^=$ supply were not significant. Likewise, the interaction between NH_4^+ and $SO_4^=$ was not significant. The yield data for the 9 treatments and the mean yields at each factor level are

presented in Table 3, showing differences for main effects. The least significant difference between means for main effects was determined to be 56 grams. From Table 3, it is evident that the decrease in mean yield produced by 88 p.p.m. NH_4^+-N as compared to no NH_4^+-N$(751.5-696.3=55.2)$ was below the level of significance by only 0.8 grams. The mean yield at 175 p.p.m. NH_4^+-N was significantly less than that at either the zero or the 88 p.p.m. NH_4^+-N.

CHEMICAL ANALYTICAL RESULTS

The results of the analyses of the leaves for N, P, K, Ca, Mg, and S are presented in Table 4. The data representing the concentration of the elements are expressed as percentage of the dry weight of the leaf tissue. The different nutrient treatments had a significant effect on the concentration of these elements in the plant.

The accumulation of magnesium in the leaf tissue was depressed by both the ammonium and the sulphate ions. In general, the magnesium content of the leaf tissue was inversely related to variations in the NH_4^+ and SO_4^+ concentrations in the nutrient solutions. The highest content of magnesium in the leaves was associated with zero NH_4^+ and zero SO_4^+ in the nutrient supply (treatment 1). The content of magnesium in both top and bottom leaves decreased progressively with each successive increment of NH_4^+. By comparison, the decrease in the magnesium content of the tissues with increasing $SO_4^=$ supply was less marked in both top and bottom leaves and less progressive in the latter. The magnesium content of the leaves reached the lowest level at high NH_4^+ and high $SO_4^=$ supply (treatment 9). The magnesium content decreased more markedly with increasing NH_4^+ at each level of $SO_4^=$ supply than with increasing $SO_4^=$ at each level of NH_4^+ supply. Thus, the general trends of the relation of each of these ions to magnesium concentration in the leaves were similar, but NH_4^+ showed the more marked negative relation with the magnesium content of the leaf tissues.

The data also show specific relationships between the concentration of NH_4^+ and $SO_4^=$ in the nutrient medium and the accumulation of some of the other elements in the leaf tissue. An increase in NH_4^+ in the nutrient supply resulted in an increase in the content of N, P, K, and S and a decrease in the content of Ca in both top and bottom leaves. An increase in $SO_4^=$ in the nutrient supply resulted in an increase in the content of S in both top and bottom leaves and a decrease in the content of Ca but had relatively little effect on the other elements.

TABLE 4.—A SUMMARY OF THE MINERAL ANALYSES OF LEAF TISSUE, EXPRESSED AS PER CENT OF OVEN DRY WEIGHT

Treatment No.	Treatment		Top leaves						Bottom leaves					
	NH_4-N	SO_4-S	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
	p.p.m.	p.p.m.												
1	0	0	4.82	0.68	4.25	4.01	0.98	0.38	2.16	0.55	4.39	5.87	1.22	0.34
2	0	118	4.73	0.75	4.83	3.26	0.94	0.43	3.67	0.67	5.91	4.75	1.21	0.50
3	0	236	4.84	0.70	5.27	3.64	0.63	0.43	3.55	0.56	5.44	5.17	0.86	0.45
4	88	0	5.92	0.99	4.62	2.34	0.75	0.55	4.69	1.03	5.55	3.11	0.96	0.46
5	88	118	5.46	0.88	5.02	2.59	0.59	0.69	4.40	0.93	5.26	3.59	0.78	0.72
6	88	236	5.55	1.01	4.40	2.09	0.48	1.06	4.30	1.06	5.51	2.93	0.70	1.27
7	175	0	6.95	0.95	5.62	1.96	0.51	0.71	6.17	1.20	7.71	2.43	0.60	1.09
8	175	118	7.12	1.03	5.16	1.77	0.47	0.71	5.86	1.11	7.74	2.39	0.60	1.22
9	175	236	7.19	0.99	4.59	1.57	0.41	0.75	5.67	1.13	7.20	2.24	0.57	1.28

REFERENCES

1. Anderson, P. J., N. T. Nelson, and T. R. Swanback. Conn. Agr. Exp. Sta. Bull. 10. Report of Tobacco Station at Windsor, 1927. 1928.
2. Garner, W. W., J. E. McMurtry, Jr., C. W. Bacon, and E. G. Moss. Sand drown, a chlorosis of tobacco due to magnesium deficiency and the relation of sulphates and chlorides of potassium to the disease. J. Agr. Res. 23 : 27-40. 1923.
3. Official and tentative methods of analysis of the Association of Official Agricultural Chemists. 5th ed. 1940.
4. McEvoy, E. T. Studies on the optimum nutrition of flue-cured tobacco. Sci. Agr. 25 : 489-496. 1945.
5. McEvoy, E. T. Response of burley tobacco varieties to ionic forms of nitrogen. Sci. Agr. 26 : 640-653. 1946.
6. McEvoy, E. T. The physiological aspect of major element nutrition on the maturity of flue-cured tobacco. Sci. Agr. 31 : 85-92. 1951.
7. Pucher, G. W., C. S. Leavingworth, and H. B. Vickery. Determination of total nitrogen of plant extracts in the presence of nitrates. J. Ind. and Eng. Chem., *Anal. Ed.*, 2 : 191. 1930.

SYNTHETIC ESTROGEN IN LAMBS

I. THE EFFECT OF DIETHYLSTILBESTROL ON WEIGHT GAINS AND CARCASS GRADES OF FEEDER LAMBS[1]

F. Whiting[2], R. D. Clark[3] and C. E. Allen[4]

Dominion Experimental Station, Lethbridge, Alta., and Chemistry Division, Science Service, Ottawa, Ont.

[Received for publication September 23, 1953]

ABSTRACT

Feeder lambs were implanted subcutaneously in the neck region with a 12 mgm. pellet of diethylstilbestrol. In comparison with untreated lambs, the following differences were observed: (*a*) increased live weight gain; (*b*) lower dressing percentage; (*c*) higher moisture in round and rib sections of carcass. There was no significant difference in the feed efficiency based on carcass weight gains. It is concluded that the increased body weight resulting from diethylstilbestrol administration is due to the higher moisture content of the tissues and to the greater proportion of offal.

INTRODUCTION

The possibility of using diethylstilbestrol (DS) to increase the rate of gain and the feed efficiency of lambs has aroused considerable interest within recent years. Significant differences have been found between untreated controls and lambs implanted subcutaneously with pellets containing 12 or 24 mgm. DS (1, 2, 6, 7, 8, 9, 10, 11, 13). Many of these authors found that the treatment reduced the dressing percentage and the carcass grades. This did not occur when progesterone was used with DS (5).

The reason for the improved feed efficiency has been reported not to lie in increased digestibility of the total ration (7, 11) or of the protein (11). Jordan and Bell (7) and O'Mary *et al.* (11) reported no difference in the N retention, contrary to Whitehair *et al.* (13) for lambs and Clegg (3) with steers, who found a significant increase in nitrogen retention when DS was implanted subcutaneously.

The difference in the carcass grades may be related to the decreased amount of fat (9, 10, 11) although this may not be a factor in long term trials. O'Mary *et al.* (10, 11) observed an increase in the amount of bone and connective tissue in DS-treated lambs after a 49-day interval but there was no difference between treated and control animals when the treatment period was extended to 84 days.

Stadleman *et al.* (12) reported that DS-treated birds had a greater dressed weight than controls at 10 weeks of age but the weight of fried chicken was the same.

In view of the somewhat conflicting reports on the advantages to be gained in employing DS for feeder lambs, a preliminary trial was conducted in 1951-52 and a more extensive one in 1952-53. The moisture contents of carcasses were estimated in the latter trial. The residual estrogen content of the carcass was determined also. The feeding trials are reported here and the assays of residual estrogen will be reported in a subsequent paper (4).

---

[1] Joint contribution from the Animal Husbandry Division and the Chemistry Division (Contribution No. 242), Canada Department of Agriculture.
[2] Animal Nutritionist, Lethbridge.
[3] Animal Husbandman, Lethbridge.
[4] Chemist, Ottawa.

PROCEDURE

The general plan of both feeding trials was as follows. Feeder lambs were allotted, on the basis of sex, age and breed, to two groups. One DS pellet (12 mgm.) was implanted subcutaneously in the neck region of each lamb in one group; the other group served as a control. All lambs were fed alfalfa hay ad lib. and a grain ration according to appetite. A mineral mixture composed of equal parts of bone meal and cobaltized salt was provided. When market weight was reached the lambs were slaughted and observations made of carcass weight and quality. Samples of shoulder muscle, of liver and of kidney fat were assayed for residual estrogens.

Some minor differences in the two experiments should be noted. In 1951-52 the grain ration consisted of: barley 60, oats 25, and dried molasses beet pulp 15; whereas in 1952-53 the grain ration components were: barley 25, oat 60, dried molasses beet pulp 10, and linseed oil meal 5. Feed consumption records were kept in the latter trial only. The first experiment began November 15, 1951, and terminated February 25, 1952, a period of 104 days. The second experiment began October 28, 1952. Some of the lambs reached market weight January 27, 1953, but others were held until February 24. The moisture content of four carcasses from each group in the second experiment was estimated by taking one-pound samples of meat and bone from the round of leg and from the 9th and 10th rib. The samples were chopped and dried to constant weight. Pooled tissues were obtained from 4 wether lambs in the first experiment and from 4 wether and 4 ewe lambs in the second experiment.

RESULTS AND DISCUSSION

The average daily gain, feed consumption, feed efficiency, dressing percentage and carcass grade are shown in Table 1. The treated animals made the greater gain. However, the treated animals had the lower dressing percentage, and hence the mean carcass weights were closely similar for both groups. This confirms previous reports (loc. cit.). The two samples of the carcass had higher mean moisture contents in the case of the treated animals (1952-53 expt.).

Feed efficiency, based on the pounds of hay and grain required for 100 lb. live weight gain, was better for the treated animals. If the calculation is made on the basis of 100 lb. carcass gain, however, assuming an initial dressing percentage of 45 per cent, the feed efficiency values are not very different—hay, 644 and 684 lb., grain, 770 and 787 lb., respectively per 100 lb. carcass gain.

Carcass grades were not improved by pellet implantation.

It was very difficult to remove the pelts from the DS-treated lambs. A similar observation was made by Jordan and Bell (7).

The general conclusion drawn from the results of these two feeding trials was that there was no advantage to be gained from implanting diethylstilbestrol subcutaneously in feeder lambs. The greater body weight gains of the treated group could be accounted for by the increased moisture and offal.

TABLE 1.—GROUP AVERAGES FOR DIETHYLSTILBESTROL IMPLANTED AND CONTROL LAMBS

	1951-52		1952-53	
	Treated	Control	Treated	Control
Number of lambs	4	4	31	31
Days fed	104	104	106	110
Initial weight (lb.)	82.0	82.2	57.9	56.9
Final weight (lb.)	124.8	105.8	110.8	100.7
Daily gain (lb.)	0.41	0.23	0.50	0.40
Warm carcass weight (lb.)	59.5	53.4	51.2	50.2
Dressing percentage	47.7	50.5	46.4	49.9
Moisture in round of leg (%)	—	—	55.0	50.8
Moisture in 9th and 10th rib portions (%)	—	—	38.8	35.1
Hay per 100 lb. live weight gain (lb.)	—	—	310	374
Grain per 100 lb. live weight gain (lb.)	—	—	365	438
Carcass grades: choice (number)	4	4	28	31
good (number)	0	0	3	0

ACKNOWLEDGMENT

Grateful acknowledgment is made to A. G. Cliffe, Viobin (Canada) Limited, St. Thomas, Ontario, for the donation of the dithylstilbestrol pellets and of the implanter which were used in the experiments.

REFERENCES

1. Andrews, F. N., and W. M. Beeson. The effect of various methods of estrogen administration on the growth and fattening of wether lambs. J. Anim. Sci. 12 : 182-187. 1953.
2. Andrews, F. N., W. M. Beeson, and Claude Harper. The effect of stilbestrol and testosterone on the growth and fattening of lambs. J. Anim. Sci. 8 : 578-582. 1949.
5. Clegg, M. T. The use of diethylstilbestrol to increase nitrogen retention. J. Anim. Sci. 11 : 758. 1952. (*Abst.*).
4. Ellis, Patricia J., C. E. Allen, and F. Whiting. Synthetic estrogen implants in lambs. II. The diethylstilbestrol content of treated lamb tissues. Can. J. Agr. Sci. 34 : 292-298. 1954.
5. Galloway, J. H., L. J. Bratzler, L. H. Blakeslee, and J. Meites. Effect of stilbestrol-progesterone implants on growth and carcass quality of lambs. Mich. Agr. Expt. Sta. Quart. Bull. 35 : 68-74. 1952.
6. Jordan, R. M. The effect of stilbestrol on fattening lambs. J. Anim. Sci. 9 : 383-386. 1950.
7. Jordan, R. M., and T. Donald Bell. Effect of stilbestrol on carcass quality and shrinkage and nitrogen retention by lambs. J. Anim. Sci. 11 : 795. 1952. (*Abst.*).

8. Klosterman, Earl W., W. E. Dinusson, Merle R. Light, and M. L. Buchanan. APF and Stilbestrol: What is their value for growing and fattening lambs. ? Bimonthly Bull. N. Dakota Agr. Expt. Sta. 14 : 43-45. 1951.
9. Means, T. M., F. N. Andrews, and W. M. Beeson. The effects of hormones on the growth and fattening of lambs. J. Anim. Sci. 12 : 176-181. 1953.
10. O'Mary, C. G., A. L. Pope, G. D. Wilson, R. W. Bray and L. E. Casida. The effects of diethylstilbestrol, testosterone, and progesterone on growth and fattening and certain carcass characteristics of western lambs. J. Anim. Sci. 11 : 656-673. 1952.
11. O'Mary, C. C., W. S. Wilkinson, G. D. Wilson, R. W. Bray, A. L. Pope, and L. E. Casida. The effect of stilbestrol on certain carcass characteristics and feed utilization of full fed and limited fed western lambs. J. Anim. Sci. 11 : 751. 1952. (*Abst.*).
12. Stadleman, W. J., C. W. McCartan, E. L. Baum, and B. A. McLaren. Growth and processing shrinkage of disthylstilbestrol fryers. Poultry Sci. 30 : 512-514. 1951.
13. Whitehair, C. K., Willis D. Gallup, and M. C. Bell. Effect of stilbestrol on ration digestibility and on calcium, phosphorus, and nitrogen retention in lambs. J. Anim. Sci. 12 : 331-337. 1953.

SYNTHETIC ESTROGEN IMPLANTS IN LAMBS

II. THE DIETHYLSTILBESTROL CONTENT OF TREATED LAMB TISSUES[1]

PATRICIA J. ELLIS,[2] C. E. ALLEN[3] AND F. WHITING[4]

Chemistry Division, Science Service, Ottawa, Ont., and
Dominion Experimental Station, Lethbridge, Alta.

[Received for publication September 23, 1953]

ABSTRACT

The change in uterine weight of weanling rats was more sensitive than the vaginal smear of spayed rats as an index of response to administered estrogen. The residual estrogen content in 100 gm. tissue from feeder lambs implanted with a 12 mg. pellet of diethylstilbestrol 15 weeks previously did not exceed 7.1 μg. in kidney fat, 2.8 μg. in shoulder muscle and 4μg. in liver tissue.

INTRODUCTION

Whiting, Clark and Allen (17) have reported the results of feeding trials in which 12 mg. pellets of diethylstilbestrol (DS) were implanted in the neck of lambs. Samples of tissue were taken at the time of slaughter and this paper records the assay of the residual estrogen content. So far as the authors are aware, similar reports in the literature are not available for lambs, although there are several reports for poultry (2, 5, 6, 16). Canadian Food and Drug Regulations now prohibit the sale of estrogenized animals for human consumption. Information on residual estrogens in the carcass is necessary before any change in the regulations can be recommended.

The residual estrogenic activity in treated poultry tissues varies according to the estrogenic agent used and the treatment interval. Jones and Deatherage (6) concluded that only insignificant amounts of DS were present. Gowe (5) reported values less than 14.5 μg. dienestrol diacetate per 100 gm. muscle and skin. Bird *et al.* (2) employing different estrogens found that tissues heavily infiltrated with lipids possessed sufficient activity for humans to induce cytological changes in vaginal smears characteristic of estrus. Vondell (16) has reported that pellets of DS may still retain appreciable estrogenic activity 20 weeks after implantation into poultry. The duration of the treatment of the lambs reported in the present experiments was from 104 to 110 days, a period longer than that usually given poultry.

Several techniques have been reported for the determination of estrogenic activity. Chemical methods have been applied to pharmaceutical preparations (13), urine (15), and cockerel tissue (6); biological methods, because of their greater sensitivity, are more generally applied to the analysis of animal tissue. The chick oviduct has been employed as an index of response* (10). Rats and mice are more commonly used as test animals. The vaginal smear method has been applied to spayed mice (4, 14) and ovariectomized rats (7, 11) although Koch (8) considered the

*Ellis, Patricia J., Chemistry Division, Science Service, Ottawa. (*Unpublished data.* 1952).

[1] Joint contribution from the Chemistry Division (Contribution No. 243) and from the Animal Husbandry Division, Canada Department of Agriculture.

[2] Assistant Chemist, Ottawa.
[3] Chemist, Ottawa.
[4] Animal Nutritionist, Lethbridge.

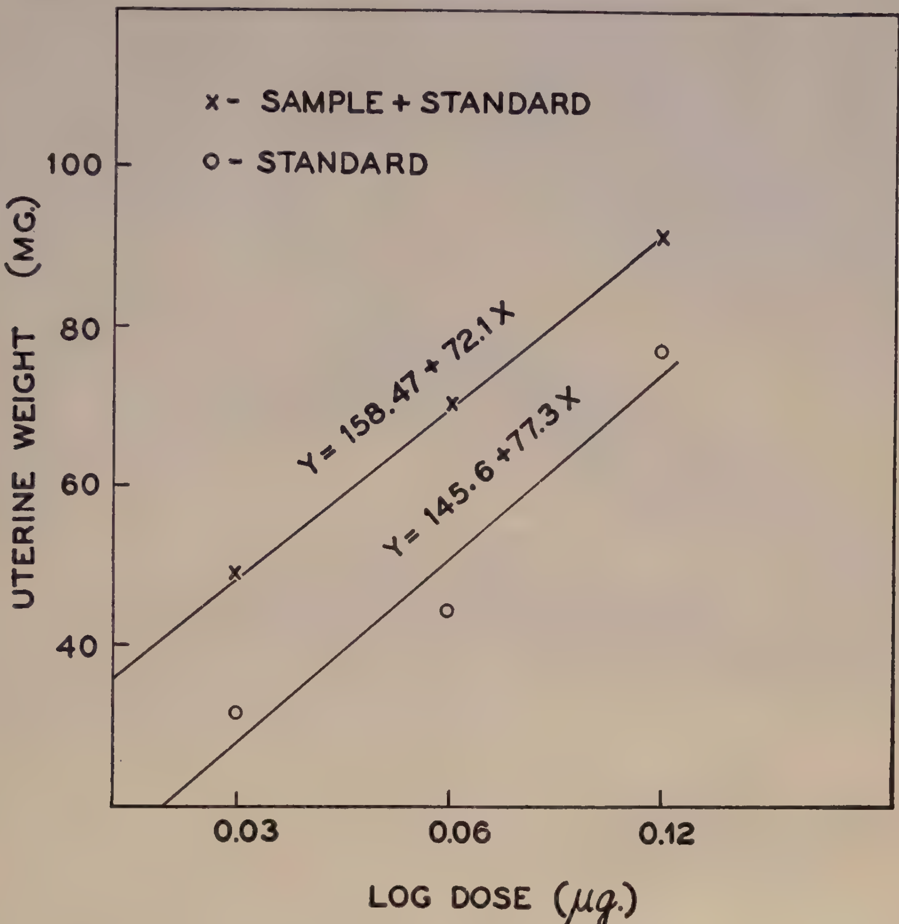


FIGURE 1. Uterine weight response to fat from treated lambs compared with diethylstilbestrol standard.

response to DS to be irregular. Several authors (1, 3, 9) have compared the precision of assays in which vaginal smears and uterine weight of spayed rats were the criteria of response. Within certain limits, increase in uterine weight is proportional to log. dose (3). Sensitivity of test animals may change (9) and frequent checks with the standard are advisable. Sealey and Sondern (12) found that spaying was unnecessary and that 0.015 μg. DS was sufficient to elicit a measurable response in the intact weanling rat. Experience in this laboratory confirms the sensitivity of this method.

PROCEDURE

Preparation of Tissue

Samples of kidney fat, of shoulder muscle and of liver were taken from the lamb carcasses at time of slaughter, frozen and shipped in ice to Ottawa. Composite samples were taken from 4 wether lambs in the 1951-52 experiment and from 8 lambs (4 of each sex) in the 1952-53

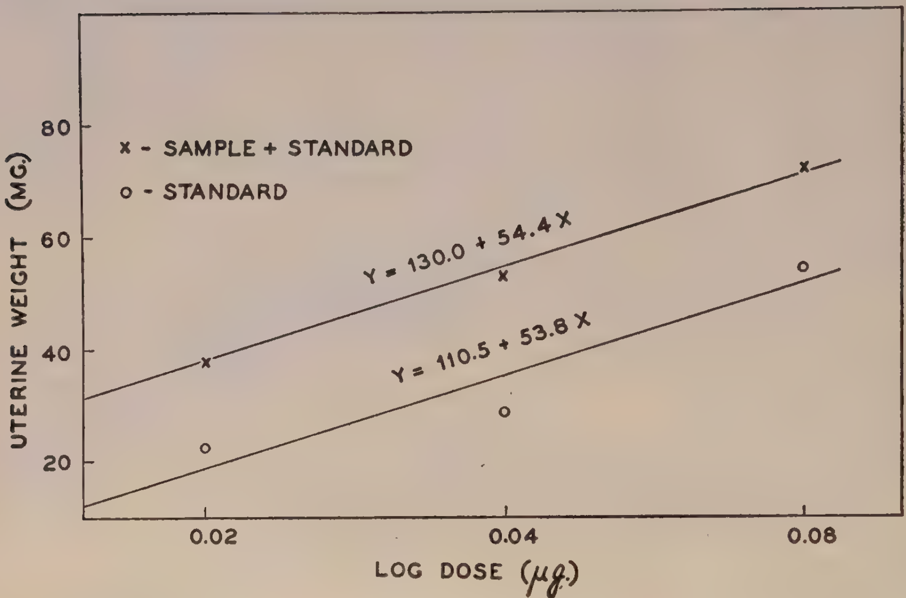


FIGURE 2. Uterine weight response to muscle from treated lambs compared with diethylstilbestrol standard.

experiment. As a check on normal tissue a composite sample of kidney fat from control lambs was included.

Fat samples were melted in a steam bath, filtered through paper in a hot water funnel and were liquefied by warming before injection into test animals. Muscle and liver tissues were ground, homogenized with alcohol in a Waring Blendor and extracted in a mixing vessel for 6 hours with 3-4 volumes of alcohol. After removal of the alcohol the tissues were extracted for a 25-hour period with ether. The solvents were removed from the combined extracts by distillation under reduced pressure.

Assay Procedure

(*a*) *Parenteral Administration*

The test animals were albino female rats 21 to 23 days of age and weighed 29 to 41 gm. Daily injections were given subcutaneously in the flank on 3 successive days. The animals were killed 24 hours after the last injection, the uteri were dissected free from adhering tissue and the intra-uterine fluid expressed on blotting paper before weighing.

DS standards of 0.02, 0.04 and 0.08 µg., dissolved in corn oil, were administered to groups of 10 to 12 weanlings. Only one level, the maximum injectable, of the test samples was injected in the 1951-52 series. By incorporating appropriate amounts of DS standard in the 1952-53 test samples, graded doses could be used. Liver tissue appeared to inhibit the response to added estrogen, and this method could not be employed with these samples.

(*b*) *Oral Administration*

Extracted residues were fed to assess the completeness of extraction. Intact weanling and spayed adult rats were used, the latter in the early

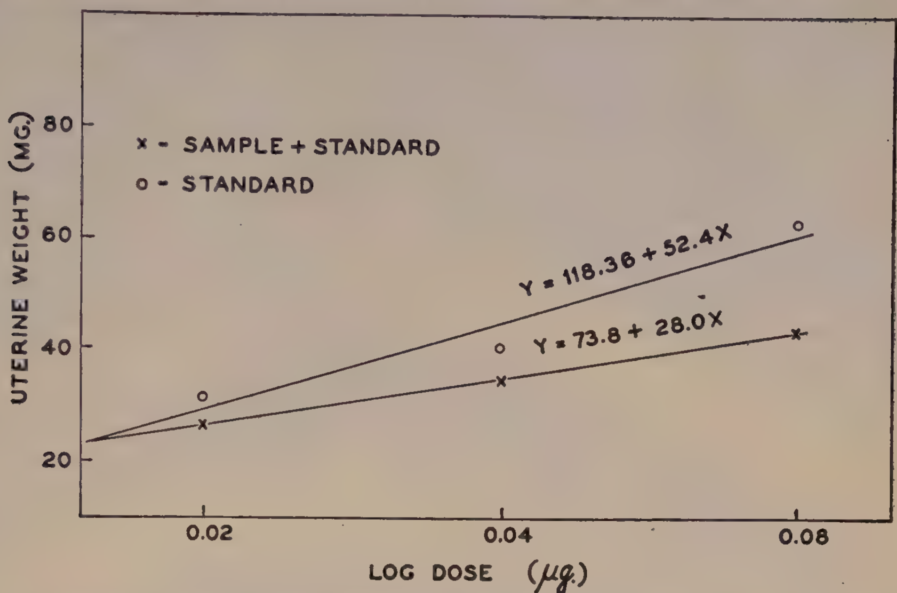


FIGURE 3. Uterine weight response to liver from treated lambs compared with diethylstilbestrol standard.

part only of the investigation. Three levels of standard DS were added as alcoholic solutions to ground stock diet, thoroughly mixed and dried. Test materials were admixed with the stock diet and sufficient corn oil was added to improve palatability. Satisfactory dosage levels of the standard were 0.45, 0.90, 1.80 μg. per 10 gm. feed in the case of spayed adults and 0.225, 0.45, 0.90 μg. per 10 gm. feed in the case of intact weanlings.

Ten to fifteen rats were allotted to each group. They were starved for 24 hours. The spayed rats were allowed to feed for 24 hours and vaginal smears were examined 48 and 56 hours later. Estrogen activity was calculated from the percentage response. Weanling rats had access to the feed mixtures for three days. Uteri were weighed on the fourth day and the potency estimated from the response obtained.

RESULTS AND DISCUSSION

The potency values of lamb tissues expressed as micrograms diethylstilbestrol per 100 gm. tissue are given in Table 1. Assay data for the second trial lot are shown graphically in Figures 1, 2 and 3.

It may be observed that the estrogen content of all tissues was small. It was not the same in the two experimental periods for any one kind of tissue. This difference in the estimate of residual estrogen activity may be more apparent than real, and may be a reflection of the precision of the different assay methods employed.

In the assay of kidney fat considerable variation occurred in uterine response and this was thought to be due to incomplete absorption of the injected material. Upon dissection fatty material was found in many rats at the site of injection, while in others fatty globules were observed in the

TABLE 1.—ESTROGENIC ACTIVITY OF WETHER LAMB TISSUES

Micrograms diethylstilbestrol (DS)* per 100 gm. tissue

Tissue	Experimental period		Dosage per lamb	Tissue extract		Residue after extraction		Total DS per 100 gm. tissue	
	1951–52, days	1952–53, days		1951–52, µg.	1952–53, µg.	1951–52, µg.	1952–53, µg.	1951–52, µg.	1952–53, µg.
Kidney fat	—	106	Nil	—	Nil	—	—	—	Nil
Kidney fat	104	106	12 mg. DS	3.0	7.1	—	—	3.0	7.1 ($P < 0.01$)
Shoulder muscle	104	106	12 mg. DS	0.4	2.8	Nil	Nil	0.4	2.8 ($P < 0.01$)
Liver	104	106	12 mg. DS	0.2	0.9	3.8	Nil	4.0	0.9

* Twelve mg. diethylstilbestrol implanted in each lamb.

intraperitoneal cavity. When the largest practicable amount of kidney fat was injected the response was so low that it was not possible to measure responses at three levels for comparison with three levels of the standard. The addition of graded amounts of diethylstilbestrol to the 1952-53 sample raised the response so that such a comparison could be made. The data thus obtained are shown graphically in Figure 1.

The increase in uterine weight caused by injected muscle extract was low also and the same procedure, the addition of standard DS, was followed as for kidney fat. The responses obtained are shown in Figure 2. Because very little of the injected material remained at the end of the test, it can be concluded that this extract was almost completely absorbed.

The injection of liver extract by itself gave a low response as did the extracts of other samples. Attempts to determine the potency of liver extract by the procedure mentioned above were unsuccessful because the estrogenic effect was inhibited when DS was added to it. This inhibition is apparently due to an inactivating substance in the liver extract and the effect was noted in several tests involving rats from two colonies. The liver is known to be a major site of inactivation of the naturally occurring estrogens and the results obtained suggest that the liver principle will also partially inactivate added stilbene derivatives. No reports of this action were found in the literature. The depression of estrogenic activity is illustrated in Figure 3. The injection of liver extract at the highest practicable level produced a small but measurable increase in uterine weight which indicated that absorption of the implanted pellet was still occurring at the time of slaughter.

In the examination of muscle and liver tissue from the 1952-53 trial it was evident that all estrogenic activity was removed by solvent extraction. No explanation is offered for the fact that an estrogenic principle was present in the 1951-52 livers after solvent treatment. It does not seem probable that the liver estrogen would be altered in form in one trial lot of lambs and not in another and so become less extractable. However, this phenomenon had been noted previously in the assay of skin and liver of birds which had received estrogenic treatment by pellet implantation. Whatever its form the residual estrogen elicits a uterine response in the test animal.

The amount of estrogenic activity in the tissues of treated lambs is small. It is interesting to note that the values are comparable to those found in this laboratory for tissue from cockerels undergoing similar treatment but for a much shorter period.

REFERENCES

1. Andersen, Ib., and K. Pedersen-Bjergaard. The accuracy of the biological standardization of estrone by the vaginal cornification method and the uterine weight method. Nord. Med. 8 : 1726-1730. 1940. *Cited in* Chem. Abst. 35 : 1845[6]. 1941.

2. Bird, S., L. I. Pugsley, and M. O. Klotz. The quantitative recovery of synthetic estrogens from tissues of birds (*Gallus domesticus*), the response of the birds' testis, comb and epidermis to estrogens and of humans to ingestion of tissues from treated birds. Endocrinol. 41 : 282-294. 1947.

3. Bulbring, Edith, and J. H. Burn. The estimation of oestrin and of male hormone in oily solution. J. Physiol. 85 : 320-333. 1935.
4. Emmens, C. W. The intravaginal assay of naturally occurring oestrogens. J. Endocrinol. 6 : 302-307. 1950.
5. Gowe, R. S. Residual estrogens in the tissues of fowl treated with dienestrol diacetate. Poultry Sci. 28 : 666-669. 1949.
6. Jones, Oscar, and F. E. Deatherage. The diethylstilbestrol content of the meat from chickens treated with this estrogenic substance. Food Res. 18 : 30-34. 1953.
7. Kahnt, Lydia C., and E. A. Doisy. The vaginal smear method of assay of the ovarian hormone. Endocrinol. 12 : 760-768. 1928.
8. Koch, F. C. A spayed rat estrogen assay method and its application to pure estrogens and urine extract. Endocrinol. 31 : 162-166. 1942.
9. Lauson, H. D., C. G. Heller, J. B. Golden, and E. L. Sevringhaus. The immature rat uterus in the assay of estrogenic substances and a comparison of estradiol, estrone and estriol. Endocrinol. 24 : 35-44. 1939.
10. Munro, S. S., and I. Kosin. The relative potency of several estrogenic compounds tested on baby chicks of both sexes. Endocrinol. 27 : 687-692. 1940.
11. Pugsley, L. I., and C. A. Morrell. Variables affecting the biological assay of estrogens. Endocrinol. 33 : 48-61. 1943.
12. Sealey, J. L., and C. W. Sondern. Comparative estrogenic potency of diethylstilbestrol, estrone, estradiol and estriol. Endocrinol. 29 : 356-362. 1941.
13. Sondern, C. W., and C. Burson. Semimicrochemical assay for diethyl stilbestrol. Ind. and Eng. Chem. 14 : 358. 1942.
14. Sulman, F. G. Intravaginal assay of estrogens. Endocrinol. 50 : 61-67. 1952.
15. Teague, R. S., and Albert E. Brown. Determination of diethylstilbestrol and its glucuronide in urine. J. Biol. Chem. 189 : 343-354. 1951.
16. Vondell, J. H. The longevity of estrogen pellets. Poultry Sci. 31 : 174-175. 1952.
17. Whiting, F., R. D. Clark, and C. E. Allen. Synthetic estrogen implants in lambs. I. The effect of diethylstilbestrol on weight gains and carcass grades of feeder lambs. Can. J. Agr. Sci. 34 : 288-291. 1954.

THE EFFECT OF CLIPPING FREQUENCY ON THE PRODUCTIVITY AND ROOT DEVELOPMENT OF RUSSIAN WILD RYEGRASS IN THE FIELD[1]

R. Thaine[2]

[Received for publication August 7, 1953]

ABSTRACT

The investigation provides information on the productivity, nutritive value, and root reserves of Russian wild ryegrass (*Elymus junceus* Fisch.) harvested at different frequencies during the first crop year.

It was found that three and five clippings produced a greater forage yield than one or two clippings. The root yield and root reserves, however, diminished with an increased number of clippings. The five-clipping treatment was the most desirable from the standpoint of protein production. The lignin content of the forage from two, three, and five clippings varied very little, suggesting that utilization intensity of Russian wild ryegrass may vary considerably without much loss in nutritive value of the forage.

INTRODUCTION

Russian wild ryegrass (*Elymus junceus* Fisch.) is well adapted to a dry northern climate such as exists in the Prairie Provinces of Canada. The grass is long-lived and nutritious (1, 7), but little is known about utilization practices which will allow production of a good yield of nutritious forage with a minimum of damage to the plant. In 1951, the results from a greenhouse study investigating the effect of clipping frequency on the species were reported by Thaine and Heinrichs (11). This paper presents the results from a similar experiment conducted in the field.

It has been generally established that heavy grazing or frequent clipping reduces root reserves, competitive efficiency, general plant vitality, and productivity of grasses (2, 3, 12, 14, and 15). However, Lang and Barnes (6) found that short season grasses, such as *Bouteloua gracilis* (H.B.K.) Lag. and *Buchloe dactyloides* (Nutt.) Engelm., yielded more when harvested frequently than when protected to the end of the season, although the mid-season grasses produced the most forage when cut only once.

The present investigation provides additional information on the productivity, nutritive value, and root reserves of Russian wild ryegrass harvested at different frequencies during the first crop year.

MATERIALS AND METHODS

Russian wild ryegrass, from a commercial source, was seeded June 15, 1950, in rows 18 inches apart on an area 32 feet by 74 feet. The soil was a clay loam of good natural fertility. No fertilizer was applied either in 1950 or 1951.

During the establishment year (1950) the grass was not cut but the dead growth was removed the following spring. The clipping treatments in 1951 were as follows:

A — Check, cut August 18 at end of experiment (one clipping).
B — Clipped May 18 and August 18 (two clippings).
C — Clipped May 18 and at six-week intervals (three clippings).
D — Clipped May 18 and at three-week intervals (five clippings).

[1] Contribution from the Experimental Farms Service, Department of Agriculture, Ottawa; part of a thesis submitted by the author in partial fulfilment of the requirements for the M.Sc. degree, University of Saskatchewan, carried out while holding a National Research Council of Canada Scholarship.

[2] Formerly Graduate Assistant, Dominion Experimental Station, Swift Current, Saskatchewan; now Graduate Student, Botany School, Melbourne, Australia.

The clipping treatments were replicated four times and randomized in each replicate. The plots were 6 feet wide and 12 feet long, plus borders. All clippings were made at a height of $1\frac{1}{2}$ inches.

One root sample 18 inches × 12 inches × 18 inches deep was excavated from the centre of each plot according to the method described by Pavlychenko (9). The amount of root material and crown plus stubble was calculated from these excavations and the material itself was used for determination of available carbohydrates. The crown and stubble included all material 2 inches above the ground surface and the transition material between stem growth and root growth below the ground surface.

Protein, lignin, and available carbohydrate analyses were made on a composite sample for each plot. The samples were prepared for analysis by grinding with a small hammer mill through a 0.5 mm. screen.

Protein content was determined by the standard Kjeldahl method (N × 6.25).

Lignin content was found by the procedure of Ellis *et al.* (4).

Total "available" carbohydrates were separated from the structural carbohydrates by hydrolysis with the taka-diastase method of Weinmann (13) using a highly purified commercial preparation known as Clarase. The digestion mixture was made up of 10 ml. of a 0.5 per cent Clarase solution, 10 ml. of an acetate buffer composed of a mixture of 4 ml. of 0.2 N sodium acetate and 6 ml. of 0.2 N acetic acid (pH 4.65) and 10 ml. of distilled water. A 0.1 gram sample of substrate was added to the digestion mixture in a large test tube and incubated for 44 hours at 37°-38° C. The tubes were then fitted with rubber stoppers equipped with capillary tubing, to minimize moisture losses, and placed in a boiling water bath 30 minutes. An aliquot of the mixture was centrifuged and 5 ml. of the supernatant fluid was made up to volume for carbohydrate determinations. Total available carbohydrate was determined by the Anthrone method as described by Neish (8). Results are calculated as percentage glucose and reported as available carbohydrate. The term "available" refers to those carbohydrates which are believed to be stored and can be used by the plant cells in time of need.

RESULTS AND DISCUSSION

The yield of leaves and stems, crowns and stubble, and roots are presented in Table 1, along with the protein and lignin content of leaves and stems and available carbohydrate content of crowns, stubble, and roots.

A significantly greater yield of leaves and stems was obtained from either three or five clippings than from one or two clippings. The root yield, however, diminished with an increased number of clippings. The drop was large and significant between the three- and five-clipping treatments. The yield of crowns and stubble followed a trend similar to that of the roots.

The per cent protein content, as well as the protein yield, increased progressively with an increase in number of clippings. The five-clipping treatment was the most desirable from the standpoint of protein production.

TABLE 1.—YIELD OF VEGETATIVE MATERIAL, THE PROTEIN AND LIGNIN CONTENT IN LEAVES AND STEMS, AND AVAILABLE CARBOHYDRATES IN CROWNS, STUBBLE, AND ROOTS OF RUSSIAN WILD RYEGRASS UNDER DIFFERENT INTENSITIES OF CLIPPING

Treatment	Dry matter—pounds/acre				Protein in leaves and stems		Lignin in leaves and stems per cent	Available carbohydrates in		
	Leaves and stems	Crowns and stubble	Roots to a 1½ foot depth	Total vegetative material	Per cent	Pounds/acre		Crowns and stubble, per cent	Roots, per cent	Stubble plus roots, pounds/acre
A — 1 clipping	1550	3880	3336	8766	7.35	114	13.05	14.32	31.10	1593
B — 2 clippings	1594	2537	2920	7051	17.00	271	8.25	18.05	30.10	1337
C — 3 clippings	1895	2666	2729	7290	20.29	384	7.79	14.69	25.51	1088
D — 5 clippings	1889	1780	1769	5438	22.69	429	7.53	11.95	21.43	592
Least sig. diff. (P = 0.05)	128	351	267	383	1.09	35	0.64	1.15	1.59	
Standard error—mean per cent	2.6	4.5	3.5	1.9	1.5	4.1	1.3	3.6	2.8	

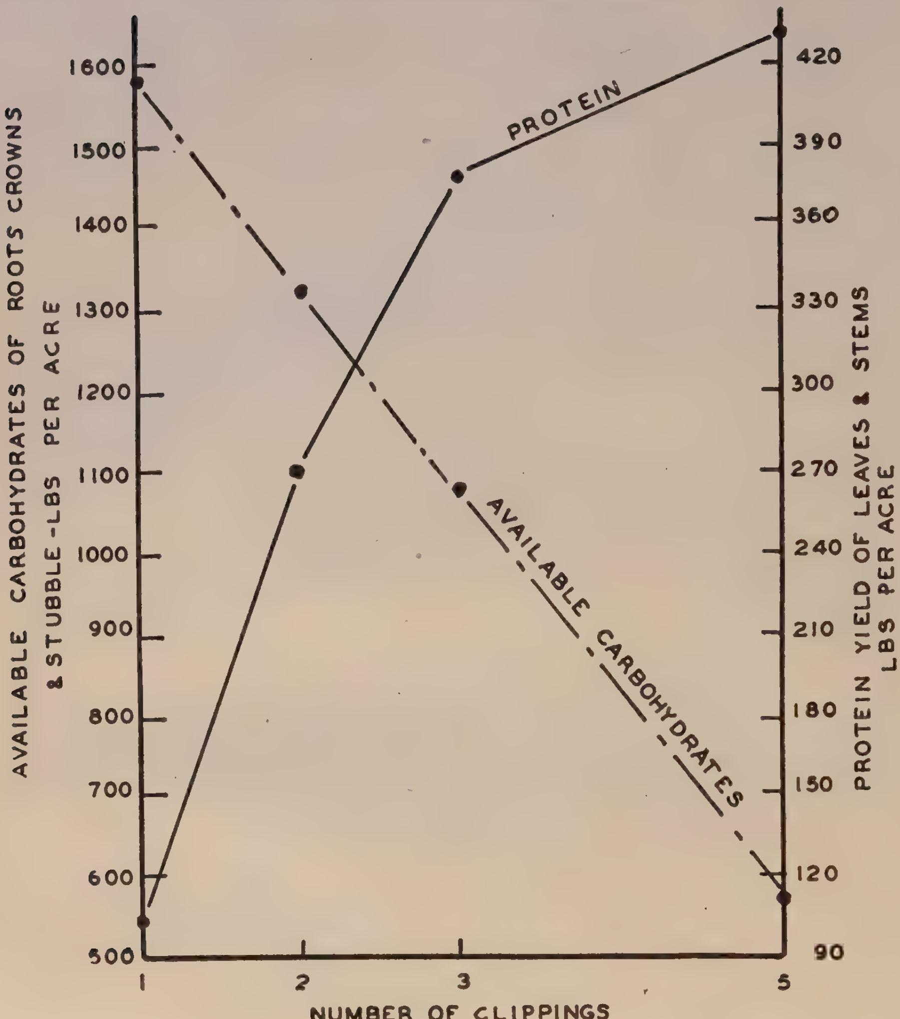


FIGURE 1. Protein yield of leaves and stems in relation to available carbohydrates in roots, crowns and stubble of Russian wild ryegrass harvested at 4 clipping frequencies.

The lignin content of the forage varied very little between the two-, three-, and five-clipping treatments, but it rose sharply in the herbage clipped at the end of the season only. This indicates that utilization intensity of Russian wild ryegrass may vary considerably without much loss in nutritive value of the forage.

The available carbohydrate content in roots, crowns, and stubble is a good indication of the amount of root reserves present. Although there was a general decline in per cent available carbohydrates from the least frequent to the most frequent clipping treatments, the root reserves still appeared to be fairly high even in the plants cut five times. The decline was about 30 per cent in the roots and about 17 per cent in crowns and stubble. However, when the total available carbohydrate production is

TABLE 2.—ROOT DISTRIBUTION OF RUSSIAN WILD RYEGRASS FOLLOWING FOUR DIFFERENT CLIPPING FREQUENCIES

Treatment	Dry matter—pounds/acre			Per cent of total yield		
	0–6 inches	6–12 inches	12–18 inches	0–6 inches	6–12 inches	12–18 inches
A — 1 clipping	1885	783	668	56.5	23.5	20.0
B — 2 clippings	1734	724	462	59.4	24.8	15.8
C — 3 clippings	1528	688	513	56.1	25.3	18.6
D — 5 clippings	995	425	349	56.2	24.0	19.8

considered the picture changes considerably. The grass when clipped five times produced only about one-half as much available carbohydrates as when clipped three times. In Figure 1 the protein yield of leaves and stems from the four treatments and the carbohydrate yield of roots, crowns, and stubble are illustrated graphically. The graph shows that increased protein production from five clippings is achieved at the expense of great loss in total carbohydrate reserves.

Root distribution observed for three depths is recorded in Table 2. The relative proportion of root material in the three soil layers (0-6 inches, 6-12 inches, and 12-18 inches) was not influenced by clipping frequency.

One of the significant features of the results was the increase in leaf and stem yield with increased clipping frequency. Most cultivated forage grasses tend to produce the maximum yield if harvested at or near the flowering stage, but Russian wild ryegrass appears to react differently in this respect and it may be that this characteristic will render it especially useful as a pasture grass.

Another desirable characteristic of the grass was the relatively high protein and low lignin content of the forage at the two-, three-, and five-clipping schedules. This would suggest that the grass could be used at a relatively wide range of utilization intensities without great differences in nutritive value of the forage occurring.

In the check treatment a yield of 2,668 pounds of root per acre foot was recorded. This yield was lower than reported by Stevenson and White (10) for crested wheatgrass and bromegrass. However, in the 12-18 inch depth more roots were produced than by any grass studied by Gist (5). It may be this ability to produce heavy root growth at lower depth that enables Russian wild ryegrass to withstand severe droughts without any apparent damage.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to G. N. Denike, Superintendent, Dominion Experimental Station, Swift Current, Saskatchewan, for his permission and encouragement to grow the plant material and carry out part of the study at the Experimental Station, and D. H. Heinrichs,

of the same institution, for his invaluable aid throughout the course of the investigation.

He is also indebted to R. P. Knowles, Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan, for his help during the preparation of the manuscript; to W. J. Pigden for advice concerning lignin and carbohydrate analyses; to F. G. Warder, Dominion Soils Laboratory, Swift Current, Saskatchewan, for conducting the protein analysis; and to M. Shaw, Plant Physiologist, University of Saskatchewan, for making available the facilities of his laboratory.

REFERENCES

1. Bell, J. M., G. H. Bowman, and R. T. Coupland. Chemical composition and digestibility of forage crops grown in Central Saskatchewan, with observations on Kochia species. Sci. Agr. 32 : 463-473. 1952.
2. Carter, J. F., and A. G. Law. The effect of clipping on the vegetative development of some perennial grasses. J. Amer. Soc. Agron. 40 : 1084-1091. 1948.
3. Clarke, S. E., E. W. Tisdale, and N. A. Skoglund. The effects of climate and grazing practices on short grass pairie vegetation. Canada Dept. of Agric. Tech. Bull. 46. 1943.
4. Ellis, G. H., G. Matione, and L. A. Maynard. Seventy-two per cent sulphuric acid method for the determination of lignin and its use in animal nutrition studies. J. Anim. Sci. 5 : 285-297. 1946.
5. Gist, George R., and R. M. Smith. Root development of several common forage grasses to a depth of eighteen inches. J. Am. Soc. Agron. 40 : 1036-1042. 1948.
6. Lang, R., and O. K. Barnes. Range forage production in relation to time and frequency of harvesting. Wyoming Agr. Expt. Sta. Bull. 253. 1943.
7. McCall, R., R. T. Clarke, and A. R. Patton. The apparent digestibility and nutritive-value of several native and introduced grasses. Montana Agr. Expt. Sta. Bull. 418. 1943.
8. Neish, A. C. Analytical methods for bacterial fermentations. National Res. Council, Prairie Reg. Lab. Report No. 46-8-3 (revised), Saskatoon, Saskatchewan. 1950.
9. Pavlychenko, T. K. The soil-block washing method in quantitative root study. Can. J. Res. *C*, 15 : 33-57. 1937.
10. Stevenson, T. M., and W. J. White. Root fibre production of some perennial grasses. Sci. Agr. 22 : 108-118. 1941.
11. Thaine, R., and D. H. Heinrichs. The effect of clipping frequency on the productivity and root development of Russian wild ryegrass (*Elymus junceus* Fisch.) in the greenhouse. Sci. Agr. 31 : 316-322. 1951.
12. Weaver, J. E. Effects of different intensities of grazing on depth and quantity of roots of grasses. J. Range Mgmt. 3 : 100-113. 1950.
13. Weinmann, H. Determination of total available carbohydrates in plants. Plant Phys. 22 : 279-290. 1947.
14. Weinmann, H. Underground development and reserves of grasses. J. Brit. Grasslands Soc. 3 : 115-140. 1948.
15. Weinmann, H. Productivity of Marandellas Sandveld pasture in relation to frequency of cutting. Rhodesia Agr. J. 46 : 175-189. 1949.

THE EFFECTS OF FROST DAMAGE ON THE NUTRITIONAL VALUE OF WHEAT[1]

J. M. Bell and Mary Gidyk[2]

University of Saskatchewan, Saskatoon, Sask.

[Received for publication October 5, 1953]

ABSTRACT

Wheats varying in bushel weight from 63 to 28 pounds cleaned, and representing various degrees of frost damage, were analysed chemically and subjected to feeding tests with mice. Aside from a decrease in energy content there were no deleterious effects on feeding value until bushel weight fell below 44 pounds. Supplementation of wheat weighing 28 pounds per bushel with starch, protein and brewers' dried yeast, in addition to minerals and fat soluble vitamins, failed to effect full restoration of feeding value as compared to sound wheat. Evidence is presented to indicate that failure in wheat of low bushel weight was due to bulkiness rather than to toxic effects or nutrient content.

INTRODUCTION

Despite the fact that frost usually damages some portion of the grain crop in Western Canada each year, no attempt appears to have been made to determine specifically what happens to nutritional quality when freezing halts the maturation process. Cereal chemists detect distinct differences in certain bread-making characteristics of wheat but these changes have not been associated with deterioration in feeding value, nor have such investigators concerned themselves with severely damaged grain such as would ordinarily be fed to livestock.

In the case of wheat at least, freezing of the immature grain reduces the endosperm or flour yield. This can be associated chiefly with starch deposition and implies a decreased energy content of the kernel. Little, however, is known about the effects on amino acid distribution (protein quality) or about the B-vitamin group, both of which are of particular importance to non-ruminants. Some reports (1, 4) indicate that the B-vitamins accumulate in the kernel very early in its development although it is not until near maturity that they translocate to the various fractions of the seed. Provided, therefore, that vitamin destruction did not result from frost damage, frozen grain may well contain more B-vitamins (on a weight basis) than does sound grain.

The experiments here reported concern the effects of frost damage on the energy, protein and B-vitamin components of hard red spring wheat harvested in 1950 following a severe early frost.

MATERIALS AND METHODS

Samples

A number of samples of wheat representing crops that had been frozen at different stages of maturity were secured. Four samples from the lot were cleaned of weed seeds, chaff and other foreign matter so that wheats weighing 57, 52, 44 and 28 pounds per bushel, after cleaning, were available

[1] Contribution from Department of Animal Husbandry.

[2] Professor of Animal Husbandry, and technician, respectively.

for study. These represented one variety and one region so that varietal and soil fertility effects would be minimized. A sample of elite seed weighing 63 lb./bu. was used as a control.

Immediately prior to the feeding trials the wheats were finely ground for incorporation into the rations and routine feedingstuffs analyses were made.

Animals

Mice of Carworth No. 1 strain were used in the feeding trials. Two male and two female weanlings were individually allotted at random to diets and to cages in a battery unit. The cages were of metal construction with wire-mesh bottoms to allow excreta to pass through. Both feed and water were provided ad libitum and the room temperature was thermostatically maintained at 28° C. The feeding tests were of two weeks' duration and the gains in body weight during this period were used to evaluate nutritional quality of the diets.

Experiment No. 1

This experiment was designed to yield information on the effects of frost on energy (starch), B-vitamin content and protein quality. A factorial design was adopted in which the five wheats were tested with various supplements of starch, brewers' dried yeast and either linseed oilmeal or skimmilk powder, so that all possible treatment combinations were made (Figure 1).

The amount of starch added to regulate the energy levels was based on the nitrogen-free extract(N.-F.E.) content of the wheat in question and on the assumption that corn starch was 100 per cent N.-F.E. Sufficient starch was added to the wheat to obtain an estimated 70 per cent N.-F.E., slightly in excess of the value for top quality wheat. Linseed oilmeal and skimmilk powder were chosen as protein supplements in order to detect variations in protein quality in the wheat. When these protein supplements were added the protein content of the rations was 20 per cent but the amount of protein in the other diets ranged between 14 and 17 per cent. Brewers' dried yeast was used at the rate of 5 per cent of the diet to supply additional B-vitamins.

All diets were adequately supplemented with minerals* and with vitamins A, D and E.

Experiment No. 2

This experiment was somewhat similar in design to the previous one but its major objective was to determine the extent to which bulkiness was responsible for the decreased feeding value of badly frozen wheat. For this purpose 63, 44 and 28 lb./bu. wheats were used, and in each case bulk was modified as follows; (*a*) nil; (*b*) cellulose** to bring the cellulose content in the 'wheat + cellulose' fraction to 7 per cent and thereby to equalize the fibre contents of the three wheats; and (*c*) agar to increase the *wet bulk* to a volume slightly exceeding that of ground 28 lb./bu. wheat after soaking in 0.9 per cent saline.

---

* *Mineral mixture:* bone meal 28, $CaCO_3$ 47, NaCl 25, Kl-calcium stearate 0.02, $FeSO_4$ 2, $MnSO_4$ 0.05, and $CuSO_4$ 0.01 gm. Added at the rate of 3 per cent of the ration.

** Solka floc BW 40, Brown Corporation, Montreal, Que.

- Wheat (63, 57, 52, 44 or 28 lb./bu.)
  - Nil
    - Nil
      - Nil
      - Linseed oilmeal
      - Skimmilk powder
    - Brewers' dried yeast
      - Nil
      - Linseed oilmeal
      - Skimmilk powder
  - Starch
    - Nil
      - Nil
      - Linseed oilmeal
      - Skimmilk powder
    - Brewers' dried yeast
      - Nil
      - Linseed oilmeal
      - Skimmilk powder

FIGURE 1. Design of Experiment 1. Two male and two female weanling mice were allotted at random to each of the 60 rations.

A number of cross-treatments were made on the nine wheat-bulk basals in order to gain more information on protein and B-vitamin adequacy, and to study the effect of an antibiotic feed supplement on the various rations. Thus all possible combinations of (*a*) nil or linseed oilmeal to give 17 per cent protein in the diet; (*b*) nil or 2 per cent *dl*-methionine; (*c*) nil or 2 per cent of a 1 : 1 brewers' yeast-alfalfa meal mixture; and (*d*) nil or 1 per cent aurofac* were obtained in the cross-treatments.

RESULTS AND DISCUSSION

Contrary to inferences that might be drawn from market grades of wheat ranging from No. 1 hard to very low quality Feed Wheat, the chemical composition shown in Table 1 reveals rather small differences in feeding value. Wheat weighing as little as 44 lb./bu., well below milling quality, closely resembled sound wheat in composition. The main effects

* Contained 1.8 gm. vitamin B_{12} and 1.8 gm. aureomycin/lb. Courtesy Lederle Laboratories Division, Pearl River, N.Y.

TABLE 1.—CHEMICAL COMPOSITION OF FROZEN WHEAT USED IN FEEDING TRIALS

Weight per bushel	Moisture	Crude protein	Crude fibre	Ash	Ether extract	Nitrogen-free extract
(lb.)	%	%	%	%	%	%
63	11.8	15.3	2.3	1.6	2.1	66.9
57	10.2	15.1	2.7	1.9	2.3	67.8
52	11.8	14.0	1.9	1.7	1.9	68.7
44	10.2	15.1	2.5	1.7	2.6	67.9
28	10.5	17.5	4.6	2.8	3.2	61.4

noted in the poorest sample were slight increases in protein, ether extract, ash and fibre contents with a corresponding decrease in N.-F.E. The Total Digestible Nutrient contents of the three wheats ranged from 79 to 71 per cent, based on the formula of Schneider *et al.* (6) for swine feeds.

Since it would appear that chemical composition does not truly reflect nutritive value, it should be recognized that composition is expressed on a weight basis whereas bulkiness may be a major determining factor, particularly as it may affect feed intake levels.

Experiment 1

The growth responses of the mice revealed no important deleterious effects in frozen wheat weighing 44 lb./bu. or more but the animals grew poorly on 28 lb./bu. wheat supplemented only with minerals and fat-soluble vitamins.

The more pertinent results of the test are shown in Table 2 from which it seems apparent that when wheat constitutes the major grain of the ration the decreased starch (energy) content does not constitute a serious problem until wt./bu. falls below about 45 lb., and even after the point is reached where poor growth results, the restoration of the energy component does not restore the nutritive value to equal that of better wheats.

Protein supplements appear to have been of relatively little value thus indicating that neither the level nor quality of protein was an important limiting factor in the wheat rations. Only in the case of 28 lb./bu. wheat did growth rates respond to a protein supplement and since the increase was similar to that obtained from starch, energy was probably the important factor involved.

A significant interaction occurred between protein and energy supplements to the various wheats. These effects are illustrated by the gains shown in the last section of Table 2 from which it can be seen that 28 lb./bu. wheat responded best to a combined supplement of starch and skimmilk powder. The superiority of skimmilk powder over linseed oilmeal may have been due primarily to its concentrated nature rather than to any particular characteristic of its protein. It will also be noted that the use

TABLE 2.—EFFECTS OF ADDING ENERGY, PROTEIN AND VITAMIN SUPPLEMENTS TO WHEAT HAVING VARIOUS DEGREES OF FROST DAMAGE. GROWTH TRIALS WITH MICE

Treatment	Wheat (lb./bu.)				
	63	57	52	44	28
	(gm. gain)				
Energy (nec. diff. = 0.9 gm.)—					
No starch added	10.8	9.6	10.0	10.0	3.9
Starch	10.5	10.2	10.2	10.4	6.9
Protein (nec. diff. = 1.1 gm.)—					
No protein added	10.4	9.3	10.2	10.5	4.5
Linseed oilmeal	11.0	10.1	9.5	10.3	4.9
Skimmilk powder	10.5	9.6	10.6	9.7	6.8
B-vitamins (nec. diff. = 0.9 gm.)—					
No brewers' yeast	10.4	10.0	9.7	9.8	5.2
Brewers' yeast	11.0	9.7	10.4	10.6	5.6
Energy and protein (nec. diff. = 1.6 gm.)—					
Nil	10.5	9.9	10.9	10.2	3.6
Linseed oilmeal	11.2	9.9	9.0	9.8	3.7
Skimmilk powder	10.8	8.9	10.0	9.9	4.3
Starch	10.4	9.9	9.4	10.7	5.4
Starch + linseed oilmeal	10.8	10.3	10.1	10.8	6.1
Starch + skimmilk powder	10.3	10.2	11.1	9.6	9.3

of brewers' dried yeast as a B-vitamin supplement had little effect on growth with any wheat, thus indicating that these vitamins are not likely reduced by frost damage.

The results of this experiment indicate that the nutritional value of frozen wheat weighing as little as 44 lb./bu. is not seriously lowered, but below that weight changes take place that are not explained by changes in chemical composition. The possibility, of course, exists that wheat of milling quality was too 'concentrated' a feedstuff for best utilization as a major ration component and that adverse nutrient changes in wheats varying from 63 to 44 lb./bu. were partially obscured by gradual improvement in the physical nature of the product.

Experiment 2

The results of this test (Table 3) confirmed the inferiority of 28 lb./bu. wheat as a feedstuff and supported the theory that the main factor involved is bulkiness. Fibre content failed in this case to reflect feeding value since the addition of enough cellulose to 63 lb./bu. wheat to exceed the cellulose content of 28 lb./bu. wheat improved the product as a feedstuff. On the other hand the addition of agar to approximate the 'wet bulk' of wheat weighing slightly less than 28 lb./bu. clearly depressed growth responses to both 63 and 44 lb./bu. wheats.

The value of added protein was somewhat dependent upon the inclusion of the yeast-alfalfa supplement. As in the previous experiment neither vitamin nor protein supplements to 63 lb./bu. wheat were effective, but a combination of the two increased gains from 8.4 to 9.8 grams. The wheat weighing 44 lb./bu. was improved by both the yeast-alfalfa and the linseed

TABLE 3.—EFFECTS OF BULK MODIFIERS, PROTEIN, VITAMIN AND ANTIBIOTIC SUPPLEMENTS ON FROZEN WHEAT RATIONS. RESULTS OF GROWTH TRIALS

Treatment	Number of mice	Wheat (lb./bu.) 63	44	28
		(gains in grams)		
All rations (nec. diff. = 0.3 gm.)	96	8.8	9.1	5.9
Bulk modifiers (nec. diff. = 0.6 gm.)—				
Nil	32	10.6	10.2	5.8
Cellulose	32	11.2	9.9	5.8
Agar	32	4.6	7.3	6.2
Protein supplements (nec. diff. = 0.5 gm.)—				
Nil	48	8.2	8.8	6.0
Linseed oilmeal	48	9.4	9.4	5.8
Vitamin supplements (nec. diff. = 0.5 gm.)—				
Nil	48	8.7	9.0	6.3
Linseed oilmeal	48	8.9	9.2	5.6
Antibiotic—B_{12} suppts. (nec. diff. = 0.5 gm.)—				
Nil	48	8.4	8.8	6.1
Aurofac	48	9.1	9.4	5.7
Protein × vitamin suppt. (nec. diff. = 0.8 gm.)—				
No linseed oilmeal, no yeast-alfalfa	24	8.4	8.3	6.6
No linseed oilmeal, + yeast-alfalfa	24	8.0	9.4	5.5
Linseed oilmeal, no yeast-alfalfa	24	9.0	9.7	5.9
Linseed oilmeal, + yeast-alfalfa	24	9.8	9.1	5.8
Methionine × protein suppt. (nec. diff. = 0.8 gm.)—				
No linseed oilmeal, no methionine	24	9.3	9.6	7.4
No linseed oilmeal, + methionine		7.1	8.1	4.7
Linseed oilmeal, no methionine		11.2	10.9	6.9
Linseed oilmeal, + methionine		7.6	7.8	4.8

oilmeal supplements with an indication that the most effective supplement was the yeast-alfalfa. It will be recalled that a trend in this direction for 44 lb./bu. wheat also existed in the first experiment so it is probable that this particular sample contained less of one or more B-vitamins than did the sound wheat. The 28 lb./bu. wheat failed, as before, to be improved by either protein or vitamin supplements.

The use of methionine consistently caused growth depression and suggested toxic effects. Methionine was included as a treatment in this experiment because a comparison of the amino acid content of wheat (3) with the dietary requirements of the rat (5) indicated that methionine was the major limiting amino acid. Lysine however was in approximately the same position relative to the nutritional requirements. There also is a distinct possibility that the amount of methionine used was unnecessarily large, particularly if the d-isomer is effective for growth as reported by Jackson *et al.* (2) and Rose *et al.* (5).

The effect of the antibiotic feed supplement varied with the quality of the wheat. Improved growth resulted from its use with 63 and 44 lb./bu. wheats but it tended to depress growth on 28 lb./bu. wheat.

TABLE 4.—GROWTH RESPONSES TO RATIONS VARYING IN 'BULKINESS' AND VARIOUSLY SUPPLEMENTED WITH PROTEIN, VITAMINS AND ANTIBIOTICS

Treatment	No. of mice per group	Bulk modifier		
		Nil	Cellulose	Agar
All rations (nec. diff. = 0.3 gm.)	96	8.9	9.0	6.0
Protein supplements (nec. diff. = 0.5 gm.)—				
Nil	48	8.9	8.8	5.4
Linseed oilmeal	48	8.9	9.1	6.6
Antibiotic—B_{12} supplements (nec. diff. = 0.5 gm.)—				
Nil	48	8.3	8.6	6.4
Aurofac	48	9.4	9.3	5.6
Protein × vitamins × antibiotics (nec. diff. = 1.1 gm.)—				
No protein, no yeast, no aurofac	12	7.8	9.6	5.4
No protein, no yeast, + aurofac	12	9.6	8.8	5.6
No protein, + yeast, no aurofac	12	8.8	7.7	5.3
No protein, + yeast, + aurofac	12	9.2	9.2	5.4
Linseed oilmeal, no yeast, no aurofac	12	9.2	8.5	7.6
Linseed oilmeal, no years, + aurofac	12	9.4	9.3	5.2
Linseed oilmeal, + yeast, no aurofac	12	7.5	9.0	7.1
Linseed oilmeal, + yeast, + aurofac	12	9.6	9.8	6.2

Owing to the difficulty of keeping accurate feed records no feed consumption data were obtained, but there were obviously no marked differences in the palatability of the wheat samples. Results of subsequent experiments, in which new-type feeders were used, supported this observation.

It is of interest to examine the effect of nutrient additions to rations varying primarily in bulkiness. The more important results are given in Table 4 which shows the effects of protein, aurofac and vitamin supplements. Since the statistical analyses revealed a significant interaction ($P = 0.01$) for protein × vitamin × antibiotic supplements, the independent results are best obtained from the last section of the table. When bulkiness was unaltered, aurofac alone, yeast + aurofac, linseed oilmeal, and linseed oilmeal + yeast + aurofac improved gains equally well thus indicating that vitamin supply or utilization was probably involved. On the other hand when cellulose was added gains increased from 7.8 to 9.6 grams and the further addition of protein, vitamin and antibiotic supplements failed to effect additional improvement. Since the cellulose was practically indigestible* it would appear that a change in the physical nature of the ingesta was as effective as the incorporation of vitamin or antibiotic supplements in improving vitamin nutrition.

Whereas the equalization of cellulose contents of wheats of various bushel weights failed to explain the differences in nutritive value, the results obtained from agar additions agreed with the hypothesis that *wet bulk* or *ingesta volume* are better criteria of bulkiness than are crude fibre

* Bell, J. M. (*To be published*).

or cellulose contents. When agar was added to adjust the bulkiness of the ingesta to correspond with the 'wet bulk' of wheat weighing less than 28 lb./bu. it tended to create protein and energy deficiencies. Neither aurofac nor yeast-alfalfa supplements were of value when bulkiness was thus modified. There is in fact an indication that aurofac caused growth depression in the presence of linseed oilmeal, a feedstuff having a high 'wet bulk' value.

In summarizing the results of these two experiments it should be emphasized that all the wheat samples were cleaned of chaff and other extraneous material. The results obtained do not therefore accurately indicate what might be expected under practical feeding conditions where the screenings remained with the grain. The objectives of these studies were to determine the effects of frost damage on the nutritional quality of the grain itself. The influence of fibrous diluents such as chaff will be reported elsewhere. So far as the grain is concerned, wheat frozen at various stages prior to maturity appears to suffer no serious deterioration in feeding value aside from a decreased energy or Total Digestible Nutrient content, until the weight per bushel falls below about 45 pounds. The better quality wheats appeared to be too concentrated and thus frost-damaged wheat may well be expected to give better feeding results under intensive use than good wheat. In mixtures with other grains however the fattening value of the wheat would likely be more closely correlated with the degree of frost damage (or weight per bushel). There was little indication of adverse changes in vitamin concentration or in protein quality resulting from frost damage.

ACKNOWLEDGMENTS

The authors wish to acknowledge the generous assistance of the Agricultural Representatives Branch, Department of Agriculture, Regina, and of the Saskatchewan Wheat Pool, who co-operated in securing samples of frozen wheat.

Financial assistance was provided by the Saskatchewan Research Council.

REFERENCES

1. Geddes, W. F., and M. N. Levine. The distribution of thiamin in the wheat plant at successive stages of kernel development. Cer. Chem. 19 : 547-552. 1942.
2. Jackson, R. W., and R. J. Block. The metabolism of cystine and methionine. II. The availability of d- and l-methionine and their formyl derivatives in the promotion of growth. J. Biol. Chem. 122 : 425-432. 1938.
3. McElroy, L. W., D. R. Clandinin, W. Lobay, and S. I. Pethybridge. Nine essential amino acids in pure varieties of wheat, barley and oats. J. Nutrition 37 : 329-336. 1949.
4. Pollock, J. M., and W. F. Geddes. The distribution of thiamin and riboflavin in the wheat kernel at different stages of maturity. Cer. Chem. 28 : 289-298. 1951.
5. Rose, W. C. The nutritive significance of the amino acids. Physiol. Rev. 18 : 109-136. 1938.
6. Schneider, B. H., H. L. Lucas, M. A. Cipolloni, and H. M. Pavleck. The prediction of digestibility for feeds for which there are only proximate composition data. J. Anim. Sci. 11 : 77-83. 1952.

THE PRODUCTION BY *PUCCINIA GRAMINIS* OF ABORTIVE PYCNIA ON WHEAT[1]

T. JOHNSON[2] AND G. J. GREEN[3]

Plant Pathology Laboratory, Winnipeg, Man

[Received for publication August 21, 1953]

ABSTRACT

In infection experiments with a culture of race 59 of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. and Henn., involving the infection of barberry and the subsequent inoculation of wheat seedlings with the aeciospores, it was observed that whereas some of the infections on wheat produced uredia others produced abortive pycnia. Paraphyses were present as in pycnia on barberry and a scanty exudate was produced but no pycniospores were present in it.

INTRODUCTION

Puccinia graminis Pers. is a long-cycle rust that normally produces pycnia (spermogonia) and aecia on certain species of *Berberis* and uredia and telia on some cereals and grasses. In the portion of the life cycle restricted to the barberry deviations from normal in spore production have been reported by Newton and Johnson (1) who, in greenhouse studies, observed the production of urediospores and teliospores on barberry in cultures that had either partially or entirely lost the ability to produce aeciospores. The presence of teliospores in association with aecia on plants of barberry infected naturally has been recorded by Critopoulos (2). No reports are known of the occurrence of pycnia or aecia of this rust on graminaceous hosts.

EXPERIMENTAL RESULTS

In the spring of 1952, plants of *Berberis vulgaris* were inoculated with pure cultures of physiologic races of *P. graminis* f. sp. *tritici*. The aeciospores that developed on these plants were used for the inoculation of wheat seedlings. In a study involving the inoculation of wheat seedlings with aeciospores of a culture of race 59, collected at Creston, B.C., in 1950, it was observed that no uredia were formed in some of the infections on the wheat leaves. At the time, it was assumed that these were merely weak infections of the uredial type that failed to rupture the epidermis, a phenomenon not uncommonly encountered in the "selfing" of physiologic races. A count was made which showed that 64 of the infections had produced uredia while 32 had not and were designated as "subepidermal".

From a second barberry plant infected with the same rust race, five separate pustules of aecia were used for the inoculation of wheat seedlings. When notes were being taken on the uredial development on the wheat plants it was observed that four of the aecial pustules had given rise to uredia whereas the fifth had produced only "subepidermal" infections of the appearance of those noted in the earlier infection study. An examination of these with a lens showed the presence on the lower side of the leaves

[1] Contribution No. 1282 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2] Officer-in-Charge.
[3] Plant Pathologist.

of numerous small, cushioned structures tipped by minute droplets of exudate—the whole suggestive of a dense mass of pycnia (Figure 1).

A free-hand section cut through one of the infected leaves (Figure 2) showed these structures to possess some of the salient characteristics of the pycnial stage of the rust: raised, pycnium-like bodies surmounted by unmistakable paraphyses. Microtome sections showed a dense concentration of fine mycelium in the raised areas but no spore-containing cavity, as in pycnia on barberry, was observed (Figure 3). The essential feature of the pycnial stage of the rust appeared to be absent: in none of the infections examined was there any evidence of the presence of pycniospores. A scanty, oil-like exudate was indeed present on the surface of all the infections but no pycniospores were detectable in it. It seems, therefore, that these structures must be regarded as abortive pycnia.

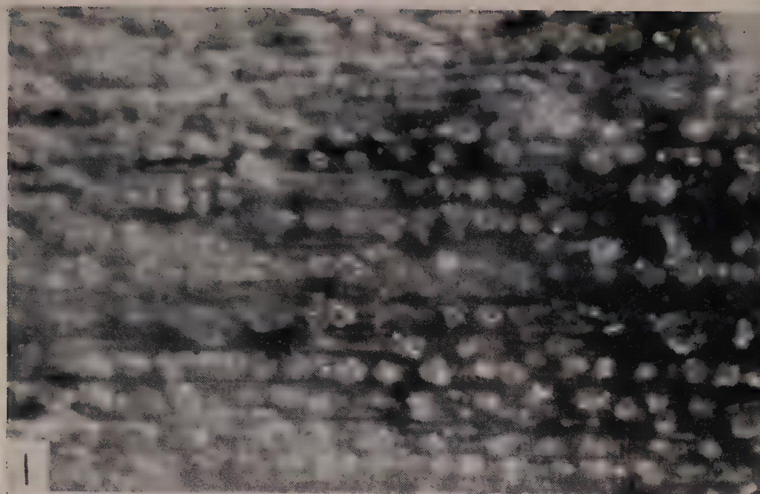
The pycnia-bearing infections on the wheat leaves were kept under observation as long as the leaves remained alive but no aecia developed. Some of the infections were given an application of pycnial exudate produced on barberry by another wheat stem rust race on the supposition that they might respond, as do similar infections on barberry, by the production of the aecial stage. Although these infections survived for almost a fortnight, there were no indications of the development of aecia.

A further test of the ability of this culture of race 59 to produce pycnia on wheat seedlings was made by infecting a third barberry plant with it and using the aeciospores for the inoculation of wheat seedlings. Only eight pustules of aecia were produced on this barberry plant and the aeciospores from only five of these produced infection on wheat seedlings. Three of these five pustules produced uredia only; one produced pycnia only; and one produced uredia and pycnia (20 infections with uredia and 17 with pycnia). As in the previous test, the pycnia-bearing infections survived for several weeks without any indication of the production of aecia or any other fruiting structures and showed no response to the application of pycniospore-containing exudate from barberry.

DISCUSSION

The above observations show that there is an inherent tendency in this culture of race 59 to produce pycnium-like structures on wheat—a tendency not present in two other cultures of the same race that were subjected to similar studies at the same time. The production of pycnia of *P. graminis* on wheat, even if these are abortive, is an indication of the existence of unsuspected potentialities in this species of rust, for there seems no good reason to suppose that whatever genetic factors are responsible for this phenomenon existed only in the particular culture studied and nowhere else in the species.

There is, at present, no knowledge of the genetical or cytological basis for this phenomenon. The examination of microtome sections of paraffin-infiltrated wheat leaves bearing abortive pycnia failed to establish definitely whether the nuclear condition was mono- or dicaryotic. It is possible that the aeciospores that gave rise to the pycnia-bearing infections were monocaryotic. The occurrence of monocaryotic aeciospores is known in some



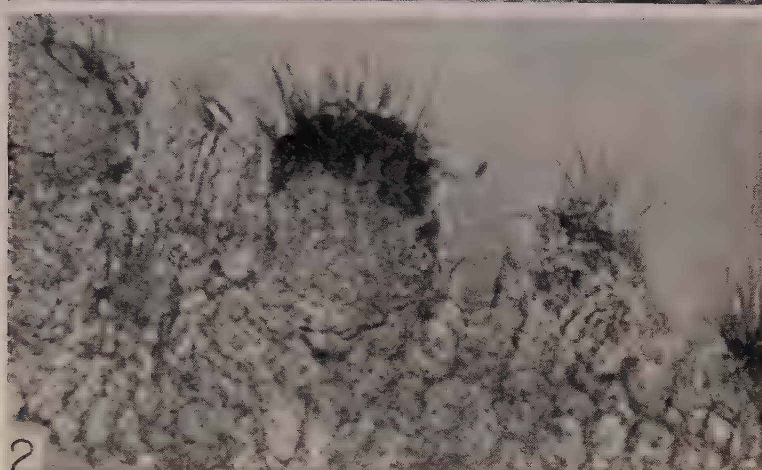


FIGURE 1. Lower surface of leaf of Little Club wheat showing pycnia (× 20).

FIGURE 2. Free-hand section of leaf of Little Club wheat showing pycnia surmounted by paraphyses (×140).

FIGURE 3. Microtome section of leaf of Little Club wheat showing aggregation of mycelium at the tip of a pycnium. Loss of epidermal cells during the sectioning process resulted in the removal of the paraphyses shown in Figure 2 (× 465).

rusts, as in *Kunkelia nitens* (Schw.) Arth., in which they function as teliospores (3). The germination of the aeciospores of those pustules of aecia that gave rise to pycnia on wheat has not been observed. If further studies on this subject can be made, attention should be given to the manner of germination and the method of host penetration.

What significance this phenomenon may have is uncertain. It seems unlikely that it could give rise to an autoecious type of *P. graminis*. Even if the pycnia should be followed by the production of either aeciospores or urediospores and these, in turn, by teliospores, the rust would not be autoecious unless the teliospores infected wheat or some other grass host instead of barberry.

REFERENCES

1. Newton, M., and T. Johnson. Production of uredia and telia of *Puccinia graminis* on *Berberis vulgaris*. Nature (London) 139 : 800. 1937.
2. Critopoulos, P. D. Production of teliospores and uredospores of *Puccinia graminis* on *Berberis cretica* in nature. Mycologia 39 : 145-151. 1947.
3. Dodge, B. O. Uninucleated aecidiospores in *Caeoma nitens* and associated phenomena. J. Agr. Res. 28 : 1045-1058. 1924.

HOUSE FLY HEAD AS SITE OF LETHAL ACTION OF DDT[1,2]

Frank O. Morrison and E. J. LeRoux

Entomology Department, Faculty of Agriculture, Macdonald College of McGill University, Macdonald College, Que.

[Received for publication September 5, 1953]

ABSTRACT

Two micrograms of DDT was applied either to the tibio-femoral membrane of one leg or to the labella of male house flies aged 4 days. Sixty-eight per cent of flies treated on the legs died within 24 hours, whereas only 31 per cent died if the cervical region had been ligated before treatment. When the DDT was applied to the labella, 83 per cent of the flies died within 24 hours, and a slightly higher proportion if the cervical region had been ligated.

When the DDT was labelled with C^{14}, 5 per cent of the radio-activity applied was recovered from the heads of unligated flies, whereas only 0.3 per cent was recovered from the heads of comparable but ligated flies. Thirty per cent of the activity was recovered from elsewhere than the heads of unligated flies treated on the labella, whereas only 2.0 per cent was recovered from elsewhere than the heads of comparable ligated flies.

Radioactive material was present in the haemolymph from the cervical region 30 seconds after application of labelled DDT to the legs. It is suggested that these facts provide evidence that: (*a*) DDT may be translocated in the haemolymph of the housefly; and (*b*) that the head is a critical region for the lethal action of DDT.

INTRODUCTION

Hoskins (1) has found DDT uniformly distributed throughout the tissues of treated insects. He suggested that his finding "might be interpreted to mean that what is important is the amount or concentration of DDT to get to a certain critical region . . ." This hypothesis, and the fact that Fisher (2) found more rapid action from DDT applied to the labella of house flies than from equal dosages applied to the tibio-femoral membranes, led the writers to the following investigation of the head as a possible "critical region".

MATERIALS AND METHODS

Four-day-old male adult house flies raised on milk (2) were used as test animals. They were immobilized and treated by the techniques developed by Fisher (2).

Carbon^{14}, labelled DDT, which had been prepared by MacDonald (3) from active sodium carbonate secured from Chalk River, and which had an activity of 2.24 millicuries per gm., was employed. Fifteen samples of 2 μgm. quantities prepared as for treatment gave an average of 342 $\pm$ 25 counts each per minute on the continuous gas flow counter (geometry 2 π). Counts were made for 5 to 10 minutes on all samples, and activities were corrected for the background. In all instances the total counts from recovered DDT or metabolites indicated a complete recovery of the applied amount. Slight day-to-day differences in counter efficiency were not serious, as all records are in terms of percentages of total recovered activity.

Groups of 5 to 20 flies were used so that recovered activity from the massed parts would be measurable. The heads and bodies of treated flies

[1] This investigation was financed by a Canadian Industries Limited Fellowship to the junior author. It is to form part of a thesis to be submitted to McGill University in partial fulfilment of the requirements for a Ph.D.

[2] Macdonald College Journal Series No. 325.

were separated after 24 hours, crushed, and extracted for another 24 hours with 2 ml. of benzol in small shell vials. Two aliquots of 0.5 ml. of extract were removed from each vial, evaporated to dryness in small stainless steel sample cups at room temperature under slightly reduced pressure, counted, and the counts averaged. The inside edges of sample cups were lined with filter paper, wet with distilled water to prevent external contamination of the cup from the benzol solution creeping over the edge.

Two μgm. doses were applied to the tibio-femoral membrane of one metathoracic leg or to the labella of each fly.

Ligatures of No. 60 cotton thread were pulled tight around the cervical region of each fly before treatment when it was desired to prevent DDT from passing to body regions on the opposite side of the ligature.

EXPERIMENTAL RESULTS

DDT was applied to the tibio-femoral membranes of 65 individual flies and small samples of haemolymph withdrawn through the cervical membranes by means of glass micropipettes. Radioactivity was detectable in all haemolymph samples even those taken as soon as 30 seconds after the application. The activity in the haemolymph rose fairly rapidly for the first 5 minutes then remained fairly constant for the next 24 hours.

The distribution of activity in the bodies of flies treated on different loci was determined 24 hours after treatment. Some flies were tightly ligated about the cervical region to impede the flow of the haemolymph. The results were as follows:

Number of flies	Ligated or non-ligated	Treated on	Percentage of total activity recovered from	
			Head	Body
18	Non-ligated	Labella	70	30
5	Ligated	Labella	98	2
20	Ligated	Labella	98	2
45	Non-ligated	Leg membrane	5	95
20	Ligated	Leg membrane	0.3	99

The mortality was checked 24 hours after the flies had been topically treated with the two microgram dosages of DDT and was as follows:

Number of flies	Ligated or non-ligated	Treated on	Percentage mortality
650	Non-ligated	Leg membrane	68
25	Ligated	Leg membrane	32
20	Ligated	Leg membrane	24
60	Non-ligated	Labella	83
5	Ligated	Labella	80
20	Ligated	Labella	90
20	Ligated	Labella	95
65	Ligated	Untreated	14

DISCUSSION AND CONCLUSIONS

It would appear that DDT or its metabolites penetrate the integument and are distributed to all parts of the fly body by the circulation of the haemolymph. This is in keeping with the recent finding of Tahori & Hoskins (6). Ligating the cervical region impeded haemolymph circulation and hence DDT distribution was slowed or prevented. Thus DDT placed on the leg of ligated flies failed to reach the head in any quantity in any number of flies. Mortality was also reduced. DDT applied on the labella likewise failed to become as concentrated in the posterior parts of the body of ligated flies as it did in non-ligated ones. Nevertheless mortality was unaffected. The possibility seems very great that the "critical region" of DDT action lies within the head of the house fly or that DDT acts on some hormone or chemical produced in the head.

REFERENCES

1. Hoskins, W. M. Recent work on the resistance problem at Berkeley. Conference on insecticide resistance and insect physiology. Nat. Acad. of Sciences, Nat. Res. Coun., Washington, D.C. Pub. 219, p. 35.
2. Fisher, R. W. The importance of the locus of application on the effectiveness of DDT for the house fly, *Musca domestica* L. (Diptera: Muscidae). Can. J. Zool. 30 : 254-266. 1952.
3. Macdonald, R., and B. E. Baker. Synthesis of C^{14} labelled DDT. Can. J. Chem. 31 : 517-519. 1953.
4. Lindquist, W. S., A. R. Roth, W. W. Yates, and R. A. Hoffman. Use of radioactive tracers in studies of penetration and metabolism of DDT in house flies. J. Econ. Ent. 44 : 167-172. 1951.
5. Lindquist, W. S., A. R. Roth, and R. A. Hoffman. The distribution of radioactive DDT in house flies. J. Econ. Ent. 44 : 931-934. 1951.
6. Tahori, A. S., and W. M. Hoskins. The absorption, distribution and metabolism of DDT in DDT-resistant house flies. J. Econ. Ent. 46 : 829-837. 1953.

A NOTE ON A SIMPLE METHOD FOR ENSILAGE PRODUCTION USING PLASTIC CONTAINERS

Increased interest in grassland agriculture has focused renewed attention upon the complex chemical and biological processes involved in the production of ensilage. Experimental work on this material has been hampered by the inconvenience and high cost involved in the use of conventional silos. In addition, such silos present sampling difficulties which, at times at least, appear to be insurmountable. To overcome these difficulties, plastic bags were used.

During August and September, 1953, lawn clippings (New Zealand Bent and Chewings Fescue) were taken directly from the catcher of a lawn-mower and 15 to 150 pounds packed by hand into plastic bags. The bags were filled to within 4 inches of the top and thermally sealed* immediately. Several types of plastic**, relatively impermeable to moisture and air, were used. The sealed bags were stored at temperatures ranging from 55° to 75° F.

Representative bags of the preserved grass were opened at intervals over a period of seven months and subjected to organoleptic examination. The grass assumed the physical appearance and odour of normal ensilage within 24 to 48 hours of packaging. During this initial interval there was no appreciable increase in temperature of the bag contents. A slight pressure developed, sufficient to distend the bag, but this was later replaced by a slight negative pressure. At this time the pH ranged between pH 5.2 and 5.4. As the storage period was lengthened, there was little or no change in the appearance of the product, although the hydrogen ion concentration increased until a pH of 4.8 was obtained after seven months. Grass clippings were subject to this method of preservation when the initial moisture content ranged from 70 to 85 per cent and the protein content from 18 to 26 per cent. The preparations were accepted with relish by sheep, beef cattle, dairy cattle and small laboratory animals.

The time of day and the moisture conditions at cutting appeared to have little or no effect on the subsequent course of events after packaging. It is well, however, to point out that packaging and sealing must be carried out immediately after the grass is cut. If a delay of an hour ensued before packaging, the normal sequence of changes giving rise to ensilage did not occur. The rapid rate of change appears to preclude the activity of bacterial agencies at this stage. This suggests that the plant enzymes played a significant role in the initial changes that took place in the contents of the sealed bags. It is suggested that this method of ensilage preparation may facilitate the study of these biochemical changes.

— W. D. KITTS,
— A. J. WOOD,
Department of Animal Husbandry,
University of British Columbia,
Vancouver, B.C.

March 15, 1954.

* Model No. DS-150-W Heat Sealer. Dobeckmun Co., Cleveland, Ohio.
** Ultron UL-7 Film, 0.008″ gauge (Monsanto Chemical Co., Springfield, Mass.) and Polyethylene, 0.004″ gauge (Bonar and Bemis Ltd., Vancouver, B.C.) are satisfactory.

NOTE ON THE EFFECTS OF LETHAL DOSES OF INSECTICIDES ON OVIPOSITION OF THE TUBER FLEA BEETLE, *EPITRIX TUBERIS* GENT. (COLEOPTERA: CHRYSOMELIDAE)

Finlayson and Neilson (1) noted that insecticides applied to potato foliage controlled adults of the tuber flea beetle, *Epitrix tuberis* Gent., without causing a satisfactory reduction in the numbers of larvae and pupae. R. Glendenning (in litt.), Officer-in-Charge, Entomology Laboratory, Agassiz, B.C., reported similar results.

Experiments were designed to determine whether the insecticides concerned stimulated oviposition, were not sufficiently toxic to prevent females from depositing their normal number of eggs before death, or were applied too late to prevent oviposition.

Since there are no external characters for separating the sexes, copulating pairs of adults were collected in potato fields near Kamloops. Ten cages, each containing a pair of copulating beetles, were arranged at random for each of 8 laboratory treatments. Each day, 4 sq. cm. of potato leaf were placed in each cage.

The insecticides, DDT, toxaphene, and calcium arsenate, were applied 24 hours after the 6-day pre-oviposition period (2): (*a*) as water solutions by dipping squares of potato leaves in the liquid and (*b*) as dusts by treating squares of green blotting-paper, 2 cm. × 2 cm., in a dust tower and placing them on the floor of the cage for the beetles to make contact without ingestion.

The solutions were as follows: 0.005 per cent DDT; 0.005 per cent toxaphene; 0.3 per cent calcium arsenate, and 0.005 per cent DDT-0.3 per cent calcium arsenate. The dusts used were 5.0 per cent DDT; 10.0 per cent toxaphene, and 69.0 per cent calcium arsenate. The amount of insecticide per square centimetre was calculated by weighing the squares of leaf or blotting-paper on a chain balance before and after treatment. The amounts of insecticide per square centimetre are given in Table 1.

Eggs were counted daily under a binocular microscope.

Although the dosage of each insecticide was lethal, oviposition did occur after treatment. Analysis of variance showed that each of the chemicals reduced oviposition significantly both from 0 to 24 hours and from 25 to 48 hours after treatment (Table 1). The highest average number of eggs laid within 24 hours after a treatment was 2.0. Almost no eggs were laid from 25 to 48 hours after treatment. No oviposition occurred after 48 hours for any treatment. Oviposition was significantly higher during the 24 hours before treatment than during the 24 hours immediately after except in the 0.005 per cent DDT-0.3 per cent calcium arsenate treatment.

All the chemicals tested produced a high mortality in a very short period (Table 1). Within 96 hours after application, 100 per cent mortality was effected by all treatments except 10 per cent toxaphene. Since all the insecticides were applied after the pre-oviposition period, the results show that relatively large numbers of immature forms appearing in the fields after the numbers of adults had been satisfactorily reduced were not due to stimulating effects of the insecticides, but probably to faulty timing of the applications.

TABLE 1.—EFFECTS OF VARIOUS INSECTICIDES IN LABORATORY EXPERIMENTS ON OVIPOSITION AND MORTALITY OF *Epitrix tuberis*

Treatment*	Amount of toxicant per square centimetre µg.	Average number of eggs laid per female						Percentage mortality		
		Before treatment	After treatment				Total	After treatment		
			0 to 24 hr.		25 to 48 hr	After 49 hr.		24 hr.	48 hr.	96 hr.
			Not transformed	Transformed**						
Untreated	0	2.6	4.7	2.15	1.9	77.7	86.9	0	0	0
0.005% DDT	0.24	4.0	1.1	1.34	0.0	0.0	5.1	55	100	100
0.005% toxaphene	0.18	3.0	1.3	1.35	0.1	0.0	4.4	75	100	100
0.005% DDT-0.3% calcium arsenate	0.19	0.7	0.8	1.27	0.1	0.0	1.6	75	100	100
0.3% calcium arsenate	1.40	2.5	2.0	1.58	0.2	0.0	4.7	50	95	100
5.0% DDT	12.00	3.5	0.1	1.04	0.0	0.0	3.6	85	95	100
69.0% calcium arsenate	83.00	2.1	0.3	1.11	0.1	0.0	2.5	75	100	100
10.0% toxaphene	10.00	3.1	1.4	1.36	0.0	0.0	4.5	55	75	95
Difference necessary for significance— 1 per cent level	—	—	—	0.74	—	—	—	—	—	—
5 per cent level	—	—	—	0.56	—	—	—	—	—	—

* All chemicals supplied by Ansell Laboratories, Vernon, British Columbia.

** The transformation $\sqrt{x+1}$ was used, x being the number of eggs laid per female.

REFERENCES

1. Finlayson, D. G., and C. L. Neilson. Experiments on the insecticidal control of the tuber flea beetle, *Epitrix tuberis* Gent., in the interior of British Columbia. Can. J. Agr. Sci. 34 : 156-160. 1954.

2. Neilson, C. L., and D. G. Finlayson. Notes on the biology of the tuber flea beetle, *Epitrix tuberis* Gentner (Coleoptera: Chrysomelidae), in the interior of British Columbia. Canadian Ent. 85 : 31-32. 1953.

— D. G. FINLAYSON,
Field Crop Insect Section,
Entomology Laboratory, Science Service,
Canada Department of Agriculture,
Kamloops, British Columbia

January 14, 1954

NOTE ON HIGH NITRATE IN SALINE AREAS

High concentrations of nitrate occur in saline areas on the Forest Nursery Station, Indian Head, Saskatchewan. Concentrations up to 400 p.p.m. of nitrogen as nitrate were found in the 0-6 inch layer and up to 4000 p.p.m. in a surface crust. The high nitrate was always associated with a high concentration of other salts, chiefly sulphates. Similar concentrations of nitrate have not been found at other saline areas.

The nitrate spots have developed in fields devoted exclusively to growing tree seedlings for the past 15 to 40 years. The seeds are planted in the fall in fallowed land and the seedlings are lifted when one or two years old. This procedure keeps the land in a more or less fallowed condition for two out of three years, a condition that favours nitrification. The available information leads to the assumption that the nitrates are leached downward with percolating moisture and then move laterally with the ground water to a point where conditions are favourable for upward movement by seepage and capillarity. Evaporation at the surface causes an accumulation of the nitrate and other salts carried in the ground water. The concentrations of salts at these points are sufficient to kill all plant growth. The presence of a water table at a depth of 4 to 8 feet has been established at several sites. Similar areas of salt concentration have not been found in adjacent fields used for the production of cereal or forage crops.

Saline areas with high concentrations of nitrate have been reported in irrigated orchards in Colorado and Utah.

Headden (1) of Colorado assumed that the high concentration of nitrate was caused by fixation of nitrogen by non-symbiotic bacteria and subsequent nitrification. Stewart and Peterson (2) of Utah did not accept this theory. Their conclusion was that the high nitrate was due to seepage water carrying nitrate which had been dissolved from country rock. Neither of these explanations seems applicable to the conditions found at the Forest Nursery Station.

This investigation is being continued.

REFERENCES

1. Headden, W. P. The fixation of nitrogen in Colorado soils. Colo. Agr. Expt. Sta. Bull. 186. 1913.
2. Stewart, R., and W. Peterson. The nitric nitrogen content of country rock. Utah Agr. Expt. Sta. Bull. 134, 1914, and Bull. 150, 1917.

— J. L. DOUGHTY,
— F. G. WARDER,
— F. D. COOK,

March 24, 1954 Soil Research Laboratory, Swift Current, Sask.

NOTE ON THE REACTION OF 2,2′-DIPYRIDYL WITH IRON IN PRESENCE OF ORGANIC MATTER[1]

The reaction with 2,2′dipyridyl has been used by Ignatieff (5), Dyer and McFarlane (3), Bloomfield (1), Schnitzer and DeLong (8), and others, for the estimation of ferrous iron in solutions containing organic matter. The present authors have observed that, in such systems, the estimates of ferrous iron content so obtained may be misleading. Results typical of our findings are presented in Table 1.

TABLE 1.—IRON CONTENT OF LEAF EXTRACTS, EXPRESSED AS MILLIGRAMS FE

	Ferrous Iron			Total iron
	Dipyridyl	Thiocyanate	Ferron	
Solution A—				
Dipyridyl colour read at once	0.046	0.005	0.005	0.136
Dipyridyl colour read after 24 hours	0.105	0.005*	0.005*	—
Solution B—				
Dipyridyl colour read at once	0.036	0.023	—	0.101
Dipyridyl colour read after 24 hours	0.084	0.023*	—	—

*These values were obtained by re-analysis of the solutions 24 hours after the first determination.

In these experiments aqueous extracts obtained from leaves of poplar (*Populus grandidentata*), collected during autumnal leaf-fall, were used. Extracts were prepared by shaking 10 gm. air-dry leaf powder with 100 ml. distilled water for one hour, filtering, and making up to a volume of 140 ml. Solution A was prepared by adding ferric alum solution to extract at pH 5.5 (maintained). Solution B was the supernatant obtained by allowing extract to react with freshly precipitated ferric hydroxide for 168 hours and centrifuging. The apparent ferrous iron contents of these solutions were estimated by measurement of the colours produced as follows:

(*a*) by the dipyridyl method (7), measuring the colour developed as speedily as possible (within 15 to 20 minutes) and again 24 hours after preparation for colour development;

(*b*) by the thiocyanate procedure (4), after adding HCl solution to a concentration of ca. N/1 to ensure liberation of ferric ions, then diluting to pH ca. 1.5 for colour development with and without addition of hydrogen peroxide;

(*c*) by the ferron method (2), subtracting the value obtained for ferric iron from the total iron content.

A dichromate oxidation of extract and of iron-enriched extract also has been used to obtain (by difference) an estimate of the ferrous iron content of the latter without formation of coloured complexes of iron. An excess of N/10 potassium dichromate solution was added to each of these extracts after acidification and addition of phosphoric acid as for estimation of ferrous iron (6). These mixtures were allowed to stand at room temperature for about 15 minutes and the excess dichromate was then titrated with

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 350.

a standard solution of Mohr's salt. The iron-enriched extract also was analysed for ferrous iron using the thiocyanate and the dipyridyl 'at once' method. The values obtained by the three procedures, in the order mentioned above, were 0.59, 0.52, and 4.23 mg. ferrous iron, respectively.

A reasonable explanation of the failure of the dipyridyl method under the conditions described is found in the effect of the sequestration of ferrous ions by dipyridyl on the oxidation potential of the ferric-ferrous couple. In a system containing oxidizable organic compounds and both ferric and ferrous ions, reduction of the activity of the ferrous ion will increase the oxidation potential of the iron couple and favour further oxidation of the organic substances with consequent reduction of ferric iron.

The results presented lead to the conclusion that the dipyridyl method should not be used for the estimation of ferrous iron in systems of the kind investigated.

REFERENCES

1. Bloomfield, C. J. Soil Sci. 4 : 5-16. 1953.
2. Davenport, W. H. Anal. Chem. 21 : 710-711. 1949.
3. Dyer, W. J., and W. D. McFarlane. Can. J. Res. 16, *B* : 91-96. 1938.
4. Houlihan, J. E., and P. E. L. Farina. Analyst 78 : 559. 1953.
5. Ignatieff, V. J. Soc. Chem. Ind. 56 : 407-410T. 1937.
6. Kolthoff, I. M., and E. B. Sandell. Text-book of quantitative inorganic analysis, pp. 608-609. Macmillan Company, New York. 1947.
7. Moss, M. L., with M. G. Mellon. Ind. Eng. Chem., *Anal Ed.* 14 : 862-865. 1942.
8. Schnitzer, M., and W. A. DeLong. Sci. Agr. 32 : 680-681. 1952.

— M. SCHNITZER,
— W. A. DELONG,
Macdonald College, Que.

April 15, 1954

NOTE ON THE VALUE OF COTTON AND PAPER BAGS FOR SELF-FERTILIZING SUNFLOWERS

In the Canadian sunflower breeding program grocery type kraft paper bags have been used to induce self-pollination, mainly because of their low cost and ease of handling in the field. In fall seasons with excess moisture they cause loss of a high proportion of the heads, due to reduction of air circulation and consequent development of moulds and rot. Another problem is the difficulty in maintaining inbred lines which set seed poorly under paper bags. Usually such lines cross readily under natural pollination or are referred to as possessing high crossability and thus potentially valuable as female parents in the production of hybrid seed.

It has been shown in both the earlier work at Saskatoon (2) and in more recent studies in Texas (1) that substantially greater amounts of seed are obtained under cotton bags than paper bags when used for self-pollinating. Thus the change to cotton bags appears desirable, providing the amount of cross fertilization is not sufficient to hamper the inbreeding progress. Kalton (1) in his Texas work found no crossing under muslin bags of 128 mesh per inch, and a negligible amount under others of 88 mesh.

The brief study reported here resembles that of Kalton's but under Manitoba conditions. Three groups of 12 plants each, of the S-37-388 inbred line or female parent of Advance, were self-pollinated using grocery type paper bags and 50 mesh cotton bags both bleached and unbleached. The bleached bags contain a white clay filler between the threads. The plants chosen were growing close to unrelated lines so that the opportunities for cross pollination were good. At harvest the seeds on each plant were counted. The following year they were grown to identify the hybrids and thus determine the amount of cross-fertilization which had occurred. The results obtained are shown in Table 1. One plant in each of the paper and unbleached bag treatments was lost between placement of the bag and harvest.

Examination of the data shows that much more seed was produced under cotton than under paper bags, the amounts being 2.7 and 6.0 times as much for the bleached and unbleached types respectively. One plant under the unbleached cotton bag had no seeds but the next lowest plant in the same treatment had 98 seeds, which is a further indication of the superiority of this bag type in the matter of quantity of seed produced.

TABLE 1.—QUANTITY, GERMINATION AND CROSS-FERTILIZATION OF SEED PRODUCED BY S-37-388 SUNFLOWER USING PAPER AND COTTON BAGS AS ISOLATORS

Bag type	No. of plants	Seeds per plant		Per cent germination	Per cent cross-fertilization
		Range	Mean		
Paper	11	5–105	46	59.2	0.3
Bleached cotton	11	7–245	123	63.3	0.5
Unbleached cotton	12	0–483	278	63.9	0.3

From the standpoint of seed quality the germination for both types of cotton bags slightly exceeds that from the paper bags. In the important feature of per cent cross-fertilization neither cotton bag shows an objectionable figure even though that from the bleached cotton is higher. In the latter instance three out of the four plants classified as hybrids for purposes of calculation were listed as probable hybrids in the records; thus the figure for the bleached cotton bags may have been lower than the 0.5 per cent shown.

The results strongly favour the adoption of cotton bags of light mesh without inter-thread filler as isolators to induce self-fertilization in sunflowers. More seed of equal or better quality is produced than when paper bags are used. Danger of loss due to mould would be less because of better air circulation. Cotton bags would be more expensive and require more time to place on the heads but this would be compensated by the lower number required to produce an equal amount of seed. Moreover, once in position they would resist weather hazards for the season. Frequently, paper bags are torn by harvest and must be replaced if loss by shattering or mixing of seed is to be avoided.

Another important consideration is that, if the relative effect of cotton and paper bags is the same on all material, then use of cotton bags may lead to production of inbred lines with greater self-sterility or conversely, greater crossability, than it has been possible to maintain with paper bags heretofore. The S-37-388 female parent of Advance hybrid frequently cross fertilizes only 45 to 60 per cent with the balance of the "hybrid" seed the result of self- or sib-pollination. It is a moderate seed producer when isolated with paper bags. If with cotton bags it becomes possible to maintain lines which would not set seed under paper bags, then female parents may be developed which will cross fertilize near 100 per cent and thus greatly improve the quality of hybrid seed.

REFERENCES

1. Kalton, Robert R. Efficiency of various bagging materials for effecting self-fertilization of sunflowers. Agron. J. 43 : 328-331. 1951.
2. Putt, E. D. Investigations of breeding technique for the sunflower (*Helianthus annuus* L.). Sci. Agr. 21 : 689-702. 1941.

— E. D. PUTT,
Experimental Station,
Morden, Manitoba

March 24, 1954

ERRATA

A number of errors occurred in printing of the paper by Albert Payette, "Gangrènes du fraisier", in the March-April, 1954, issue (Vol. 34, No. 2). These are:

Page 187, line 4: Should be "195*3*".
Page 188, line 6: Should be "verticillium".
Page 188, line 38: Should be "et qui".
Page 190, line 13: Should be no period after "Sept".
Page 193, Part IV: Omit space between lines 12 and 13, and insert period after "*Zythia fragariæ.*"
Page 194, Part IV, line 14: Should be "Linoculum".
Page 194, Part V, line 15: Should be "distillée".
Page 194, Part V, line 20: Should be "succombé".
Figure 6, facing page 195, is upside down.

In the article entitled "Note on evaluating populations of the sheep ked, *Melophagus ovinus* (L) (Diptera: Hippoboscidae), on feeder lambs", by W. A. Nelson and S. B. Slen, published in Vol. 33, No. 6, the second last sentence of the last paragraph should be deleted and the last two columns of Table 1 should read as follows:

Regression Coefficient	Standard Error of Estimate
(b_{yx})	(S_y)
.640	32
.186[1]	10
.116[1]	6
.098[1]	8

[1] The differences between these regression coefficients were not significant.